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ACTIVATION AND DIFFUSION AS FACTORS INFLUENCING THE RELIABILITY OF THE HISTOCHEMICAL METHOD FOR ALKALINE PHOSPHATASE*

By ELIZABETH H. LEDUC and EDWARD W. DEMPSEY†
Department of Anatomy, Harvard Medical School, Boston, Massachusetts

INTRODUCTION

The histochemical method for alkaline phosphatase, originally devised by Gomori (1939) and by Takamatsu (1939), has been widely employed in histology. With this method, the amount and distribution of phosphatase have been observed to change in a number of physiological and pathological states. Consequently, the method is of value since it provides an index whereby changes in the activity of tissues can be assessed. Moreover, there seems to be little doubt concerning the chemical specificity of the method, since it utilizes an enzymatic hydrolysis of an organic phosphate ester. Questions have been raised, however, concerning the limits of microscopical precision of which the method is capable. Several critical studies (Danielli, 1946; Lison, 1948; Martin & Jacoby, 1949; and Gomori, 1950) have been particularly concerned with a gradient of apparent reaction which occurs in regions neighbouring loci of high activity, especially in preparations incubated for long periods of time. This appearance suggests that the gradient may be an artefact resulting from diffusion of the enzyme or its products into the surrounding tissues.

We have attempted to study the diffusion of phosphatase from a section containing loci of high activity into a superimposed section of little or no activity. We have also investigated the affinity of tissues for phosphatase by soaking sections in solutions of phosphatase at the pH optimum for the reaction as well as at other pH values. We have also considered the possibility that activators of phosphatase may occur in tissues, and that, diffusing from regions of high concentration, they may reveal 'masked' phosphatase and thus account for the apparent diffusion of the enzyme. The results of these investigations, together with a re-appraisal of the validity and limits of precision inherent in the method, are presented in the following sections.

MATERIAL AND METHODS

All tissues were obtained from normal, untreated adult animals. The livers of four guinea-pigs were used for the study of inherent histochemically demonstrable alkaline phosphatase. Tissues selected for their high content of alkaline phosphatase include a duodenum of a rat and a human placenta.

The animals were killed by decapitation, and blocks of tissue were fixed for 24 hr. in chilled 80% alcohol. The tissues were embedded in paraffin and sectioned at 5μ. They were incubated in phosphate substrates at pH 9·4, 37° C., for 3, 6 and 24 hr. and in a few instances for 48 hr. The substrates employed include sodium glycero-

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† Present address: Department of Anatomy, Washington University, St Louis, Missouri.

phosphate, yeast nucleic acid, fructose diphosphate and adenylic acid.* The procedures followed were those used by Dempsey & Deane (1946). The sites of enzymatic activity were made visible by using cobalt nitrate and yellow ammonium sulphide to produce brown cobalt sulphide. No celloidin and no counterstain were used.

Three procedures for the activation of enzymes were employed: (1) the addition of 0·0015 M-MgCl to the incubating medium (Morse & Greep, 1947); (2) soaking the sections for 24 hr. in a 0·04 % barbital solution, pH 9·4, at room temperature prior to incubation (Greep, Fischer & Morse, 1947); and (3) treating the sections with 0·01 % trypsin in a 0·04 % barbital solution at pH 9·4 for 3 hr. at room temperature followed by incubation with or without magnesium ions (Fischer & Greep, 1948).

To inactivate the enzyme without destroying it the sections were placed in a solution of equal parts of 20 % sodium citrate and 2 % formic acid at pH 5·0 at room temperature for 3 days (Greep *et al.* 1947). For the complete destruction of the phosphatase the sections were heated in distilled water at 90° C. for 5 min. After such treatment no phosphatase activity could be demonstrated even with the use of activators. To remove nucleic acids as well as inherent phosphatase from the tissue the sections were placed in 0·1 N-HCl at 37° C. from 12 to 18 hr. (Dempsey, Singer & Wislocki, 1950).

The affinity of various tissue components for the enzyme, alkaline phosphatase, was studied by soaking sections of guinea-pig liver in solutions of the enzyme. Some of the sections received no preliminary treatment before immersion in the phosphatase solutions, whereas others were first heated in distilled water at 90° C. for 5 min. in order to destroy all intrinsic enzymes. The preparation of alkaline phosphatase employed was extracted from bovine liver (fraction C_2) according to a method shortly to be described by Cohn, Surgenor, Greene, Norberg, Derouaux, Nitschmann & Hunter (in preparation).† A 1 % solution of the powdered fraction was made in distilled water buffered with acetate and barbital buffers to pH levels of 3·5, 5·0, 7·0, 9·5 and 10·5. The alkaline phosphatase of this fraction is not completely soluble; its greatest solubility is in the more alkaline solutions. Tissue sections were soaked in these solutions containing phosphatase for 24 hr. at room temperature, then rinsed thoroughly in distilled water, to remove any phosphatase that was not bound to the tissue, and incubated with glycerophosphate and nucleic acid substrates, with or without magnesium, at pH 9·4, 37° C., for 24 hr. It is important that celloidin should not be used in this type of study, because the reaction is obscured by the deposition of phosphatase in the celloidin film at pH 5·0, 7·0 and 9·5.

OBSERVATIONS

Alkaline phosphatase in guinea-pig liver. Gomori (1941), Bourne (1943), Zorzoli & Stowell (1947) Martin & Jacoby (1949), have reported that alkaline glycerophosphatase is absent in the liver of the guinea-pig even after periods of incubation up to 24 hr. This finding is surprising in view of the ease with which phosphatase can be demonstrated in the livers of other animals. It occurred to us that the histochemical demonstration of phosphatase in guinea-pig liver might require the use of enzyme

* Sodium glycerophosphate (mixture of α and β forms) was obtained from J. T. Baker, Phillipsburg, N.J., the other substrates from the Schwarz Laboratories, New York City.

† We are indebted to Dr D. M. Surgenor, Department of Physical Chemistry, Harvard Medical School, who very kindly supplied the fraction of bovine liver phosphatase.

activators or of other changes in procedure. We have, therefore, investigated the effects of still longer times of incubation, of enzyme activators and of different substrates.

When glycerophosphate was employed as the substrate, the liver reaction was negative after incubation periods of 3–24 hr. (Pl. 1, fig. 1) except in the cytoplasm of isolated polymorphonuclear leucocytes distributed at random in the sinusoids throughout the lobules. After 48 hr. of incubation, however, in addition to an intense reaction in the leucocytes there was a moderate reaction in the nuclei of hepatic cells and of Kupffer cells in the central zone of the lobule. In this zone, also, some activity was exhibited in the cytoplasm of all elements (endothelial cells and Kupffer cells) lining the sinusoids. The positive reaction in the nucleus was confined to the nuclear membrane, the chromatin threads and the nucleolus.

The phosphatase reaction occurred prominently in the polymorphonuclear leucocytes in the sinusoids and large blood vessels with all substrates and with even the shortest incubation periods. The distribution of alkaline phosphatase in the other elements of the liver varied, however, with the different substrates. After 3 hr. incubation with fructose diphosphate and adenylic acid the leucocytes stained exclusively. After 24 hr. in the former of these substrates the smooth muscle coats of all blood vessels, the nuclei of hepatic cells and Kupffer cells in the central and middle zones of the lobule, and the nuclei of connective tissue cells in the portal canals stained light brown (Pl. 2, fig. 5); after 48 hr. all nuclei, including those of the endothelium, bile duct epithelium and portal parenchyma were stained. Adenylic acid differed from fructose diphosphate in that after 24 hr. of incubation a strong reaction (dark brown stain) was exhibited in the smooth muscle coats of arteries and in the nuclei of only endothelial and connective tissue cells (Pl. 2, fig. 6). The most pronounced reaction was demonstrated with the use of nucleic acid as substrate. Polymorphonuclear leucocytes and smooth muscle cells were positive in 3 hr., and after 24 hr. a strong reaction was found in all nuclei as well (Pl. 2, fig. 7). Longer incubation in this substrate revealed no additional sites of enzyme activity. No alkaline phosphatase was present in the bile canaliculi, in contrast to various other species (Gomori, 1941; Hard & Hawkins, 1950). Cytoplasmic phosphatase activity was high only in polymorphonuclear leucocytes and smooth muscle cells.

Activation of alkaline phosphatase. The intrinsic phosphatase of the guinea-pig liver which was inactivated in an acid solution (pH 5·0) of equal parts of 20 % sodium citrate and 2 % formic acid became reactivated by a subsequent immersion of the sections for 24 hr. in an alkaline solution (pH 9·4) of sodium barbital prior to their incubation with the phosphate substrates. The acid inactivated sections exhibited no phosphatase activity whatsoever after 24 hr. incubation with the nucleic acid substrate, while in those reactivated in the alkaline solution all tissue elements except the smooth muscle cells were stained as intensely as the control, non-inactivated sections. The smooth muscle, although positive, was lightly stained. The reactivating effect of the barbital solution was accentuated slightly by the use of a second phosphatase activator, magnesium chloride, which was added to the incubating medium, but magnesium alone, without preliminary treatment with the barbital solution, failed to restore the phosphatase activity in the acid inactivated sections. In sections of the guinea-pig liver that had not been inactivated, on the other hand, the phosphatase reaction was intensified by magnesium. The picture obtained after

incubation for 24 hr. in either the glycerophosphate or nucleic acid substrates plus magnesium was equivalent to that obtained after 48 hr. of incubation in the same substrates without magnesium (Pl. 1, fig. 2). Similar activation of intrinsic alkaline phosphatase was effected also by preliminary exposure of the sections to 0·01% trypsin at pH 9·4 for 3 hr. Subsequent incubation in the glycerophosphate and in the nucleic acid substrates without magnesium for 24 hr. gave results essentially like those of control slides after 48 hr. incubation without magnesium and after 24 hr. incubation with magnesium (Pl. 1, fig. 3). The combined effects of trypsin plus subsequent immersion in an alkaline buffer solution plus the addition of magnesium to the incubating medium gave the most pronounced intensification of activity (Pl. 1, fig. 4).

The affinity of tissues for phosphatase. The combination of alkaline phosphatase with tissue components varies according to the pH of the phosphatase solution. Sections of guinea-pig liver that had been heated to 90° C. in distilled water in order to destroy all intrinsic enzymes were placed in buffered 1% solutions of phosphatase for 24 hr. As subsequently revealed by incubation in glycerophosphate and in nucleic acid substrates with and without magnesium at pH 9·4, alkaline phosphatase had combined with the tissues most readily in the phosphatase solution at pH 5·0, to a lesser degree at pH levels of 3·5 and 7·0, and not at all at pH 9·5 and 10·5.

It was the nuclei that exhibited the greatest affinity for the phosphatase (Pl. 2, fig. 8); after immersion in phosphatase solution buffered to pH 5·0, they subsequently exhibited a dark brown stain in the nucleoli, chromatin network and nuclear membrane; after exposure to phosphatase at pH 3·5 these structures subsequently stained a very light brown, and at pH 7·0 only the nucleoli acquired a faintly demonstrable phosphatase reaction. The cytoplasm of all cells became lightly and uniformly tinged with brown after preliminary exposure to a phosphatase solution at pH 5·0 but remained negative at the other pH levels. The cytoplasm of polymorphonuclear leucocytes and of smooth muscle cells remained negative; thus, cytoplasmic loci of intense intrinsic activity showed no specific affinity for phosphatase. The same results were obtained when the sections were first treated with 0·1 N-HCl in order to remove both inherent phosphatase and nucleic acids from the tissue.

Gomori (1950) similarly exposed inactivated tissues to solutions of phosphatase and subsequently incubated them in routine manner. His results differed from ours, however, in that only a small number of irregularly distributed nuclei showed a reaction and smooth muscle and connective tissue were intensely stained. We have no explanation for these differences. The methods employed differed, since Gomori dehydrated the tissues and coated them with collodion after the immersion in phosphatase solution and prior to incubation, whereas our tissues were merely rinsed and immediately incubated. Gomori does not state the pH of his phosphatase solution.

Sections of guinea-pig liver that had not been heated and, therefore, in which inherent enzymes were preserved were also exposed to phosphatase solutions at pH 3·5, 5·0, 7·0, 9·5 and 10·5. After exposure to the solutions at pH 5·0 in particular, and also at pH 3·5 and 7·0, the sections exhibited a more pronounced reaction than the control sections that were not immersed in phosphatase solutions. This increased

activity would seem to be the result of a superimposition of the bovine phosphatase on that already present in the tissue.

Exposure of unheated sections to the phosphatase solution at pH 9·5 had a slight activating effect on phosphatase activity in the section. In order to ascertain that this effect could be attributed to the activating properties of the alkaline solution (sodium barbital buffer) in which the enzyme was dissolved, a comparison was made of the effects of exposing unheated sections: (1) to a solution of the active phosphatase, (2) to a solution of the phosphatase which had been rendered inactive by heating to 90° C., and (3) to a solution of the barbital buffer alone, all at pH 9·5. The effects of all three treatments were the same, namely, a slight activation of the intrinsic phosphatase of the tissues.

After immersion in phosphatase solutions buffered to pH 10·5, there was subsequently little or no change in the intensity of reaction in leucocytes but a diminution or complete loss of activity occurred in hepatic cells. It seems probable that some of the intrinsic phosphatase of the tissue section was dissolved out, inasmuch as alkaline phosphatase is soluble in highly alkaline solutions.

Diffusion phenomena demonstrated with superimposed sections. Sections of duodenum known to contain large amounts of alkaline phosphatase were superimposed on sections of guinea-pig liver and the slides incubated with glycerophosphate for 24 hr. This long period of incubation was used to accentuate any diffusion which might occur. Those parts of the liver that were not covered by the duodenum showed a reaction only in the polymorphonuclear leucocytes. This is the usual distribution observed after 24 hr. incubation in glycerophosphate. In the portion of liver lying directly beneath or adjacent to the strongly reacting duodenal epithelium, however, a positive reaction was evident in both the nuclei and the cytoplasm of hepatic and endothelial cells. The extent of staining in these adjacent liver cells varied according to the zones of the liver lobules involved. Where portal parenchyma was contiguous to the duodenal epithelium only two to three cells beyond the edge of the superimposed section were positive (Pl. 3, figs. 9, 12), while, on the other hand, where centrolobular cells were adjacent to the superimposed duodenal cells a broad reacting zone was demonstrated (Pl. 3, figs. 9, 11). The entire central half of a lobule stained whenever any portion of it was near the superimposed duodenum. These central cells stained in the same manner as those in which phosphatase was activated with magnesium or trypsin or by prolonged incubation.

When the underlying liver section was first heated to destroy intrinsic phosphatase, the nuclei under or immediately adjacent to the superimposed duodenum were stained (Pl. 3, figs. 10, 13). This reaction was limited to a narrow zone, one or two cells in width, in both portal and central portions of the hepatic lobule. In this instance only the nuclei were stained; the cytoplasm of all cells, including those that had previously contained large quantities of intrinsic phosphatase, remained negative. On the other hand, in a liver section in which the enzyme had been inactivated but not destroyed, in addition to the nuclear reaction, the pronounced activity in the cytoplasm of the polymorphonuclear leucocytes was also restored in the areas adjacent to the superimposed duodenum. When the procedure was reversed and the inherent phosphatase was destroyed in the duodenum instead of the liver,

no nuclear reaction could be found in the liver. In all of these experiments with superimposed, phosphatase-rich sections corresponding results were obtained when sections of human placenta were employed in place of the duodenum.

DISCUSSION

The experiments described in this paper were based on the demonstration by Martin & Jacoby (1949) that a process of diffusion takes place during incubation of tissue sections in the histochemical technique for alkaline phosphatase. These investigators superimposed phosphatase-rich sections of duodenum on sections of guinea-pig liver in which there was little or no demonstrable phosphatase and found an apparent reaction in hepatic nuclei that were adjacent to the section of duodenum. We have repeated Martin & Jacoby's experiments and confirmed their results. We have also investigated the possibilities that the diffusible substance may be the enzyme itself or an activator of the enzyme, possibilities that have been suggested by several investigators (Danielli, 1946; Lison, 1948; Martin & Jacoby, 1949; Gomori, 1950).

The observations reported in this paper suggest that the diffusion phenomena associated with the histochemical reaction for alkaline phosphatase may represent, in part but not entirely, the diffusion of activating agents. When sections of duodenum of known high phosphatase content were superimposed on sections of guinea-pig liver, an apparent diffusion of reactive material from the duodenal epithelium to the underlying hepatic tissues occurred. The extent of this diffusion varied, however, in reference to different regions of the lobules that were adjacent to the duodenal cells; thus, for example, when glycerophosphate substrate was employed a wider positive area appeared in the centrolobular zones than in the portal zones. This result bears comparison with the effect of the activation of the intrinsic phosphatase of the guinea-pig liver by treatment with alkaline solutions, magnesium or trypsin. In activated sections incubated in glycerophosphate it is in the centrolobular cells that intrinsic phosphatase activity is found.

Further suggestion of a diffusion of an activating agent from the duodenal mucosa is offered by the use of an underlying liver section in which the intrinsic phosphatase has been inactivated but not destroyed by prolonged exposure to an acid solution. The enzymatic activity of the liver is restored in those areas adjacent to the superimposed duodenal mucosa, and the distribution and intensity of activity is the same as that in parallel sections reactivated by known phosphatase activators.

That substances capable of activating alkaline phosphatase are present in tissues has been demonstrated. An activator of both cytoplasmic and nuclear alkaline phosphatase, but especially of the latter, has been found in dialysates of autolysed rat intestine and other organs by Newman, Feigin, Wolf & Kabat (1950). Furthermore, the diffusible fraction of dialysed alkaline phosphatase and a solution of phosphatase that has been boiled are both capable of reactivating alkaline phosphatase (Roche & Nguyen-Van Thoai, 1950). Thus, an activating agent that is present in tissues may be associated primarily with the alkaline phosphatase fraction itself and, assuming that this factor persists in fixed tissues, it might diffuse from sites of high phosphatase concentration into surrounding cells.

An attempt to demonstrate the diffusion of an activator in the absence of active phosphatase by mounting sections of duodenum, in which phosphatase has been destroyed by heat, upon untreated guinea-pig liver sections was not successful. During exposure to distilled water at 90° C. the activating substances may have been either destroyed or dissolved out of the sections.

In spite of an apparent activation of intrinsic phosphatase around loci of high phosphatase content, the results of superimposition of duodenum sections upon heated liver sections indicate that other factors are at work. In this instance there was a diffusion of some stained material from the phosphatase-rich duodenal epithelium into the nuclei of the subjacent hepatic cells in which there was presumably no enzyme left to be activated. The picture obtained was like that reported by Martin & Jacoby (1949) and Gomori (1950). The extent of diffusion in these heated sections was too limited, however, to account for all of the activity observed when unheated sections were employed. In both portal and central zones of the lobules, the reaction appeared in only a narrow border, one to two cells in width, immediately adjacent to the superimposed duodenal mucosa. On the other hand, the entire central half of a lobule was positive in unheated liver when a portion of it was covered by the duodenum. The distribution of the reactive material also differed in heated and unheated liver sections. In the former, only the nuclei were stained; in the latter, both nuclear and cytoplasmic elements were positive, and these in the same manner as in activated sections. Newman *et al.* (1950) found that nuclear phosphatase (their group III enzyme) was less readily inhibited by heat (10 min. at 80° C.) than cytoplasmic phosphatases. Since we were unable to reactivate phosphatase in sections that had been heated for 5 min. at 90° C., we felt that all enzymes had been destroyed. It would appear, therefore, that in addition to the diffusion of a substance that will activate inherent enzymes of tissues, there may also be a migration of reactive material from sites of intense phosphatase activity to nearby structures, specifically to nuclei, which appear to have an affinity for that substance.

We are confronted by the problem of the nature of this second diffusible substance. Whether it is the enzyme itself or the products of its activity has been considered by other investigators. Danielli (1946) and Lison (1948) have attributed the effect to the migration of the phosphatase itself in poorly fixed tissue. Feigin, Wolf & Kabat (1950) suggest that post-mortem changes occurring before fixation may be responsible for its migration. Gomori (1950), on the other hand, has presented evidence against diffusion and adsorption of phosphatase as the explanation for false localization of activity. He ascribed the effect to the migration of calcium phosphate. Martin & Jacoby (1949) discussed all three alternatives, the enzyme, its product or its activators, as possible diffusing material, but they await further experimentation before reaching a decision. While we cannot give a definite answer to this question, some pertinent observations have been made. Sections in which intrinsic phosphatase had been destroyed by heat were exposed to solutions of phosphatase buffered to various pH levels. Subsequent incubation at pH 9·4 revealed that the deposition of alkaline phosphatase in the hepatic nuclei occurred most prominently at pH 5·0 to a lesser degree at pH 3·5 and 7·0 and not at all at pH 9·5 and 10·5. It is evident from these experiments that alkaline phosphatase, even when present in high concentration in the surrounding medium, does not combine with tissue elements at

pH 9·4, the optimal pH for histochemical demonstration of phosphatase activity. We believe, therefore, that the false positive reaction obtained in our experiments with superimposed sections is not the result of the diffusion of phosphatase.

The migration of calcium phosphate, the product of this histochemical reaction, is an attractive explanation for the false nuclear staining which we have observed, but we have no direct evidence for such an explanation. Danielli (1946) has demonstrated that nuclei have a strong affinity for calcium phosphate which is liberated by non-enzymatic hydrolysis in an incubating solution buffered to pH 9·3. Gomori (1950) has described the production of false nuclear reactions in three types of experiments, including the use of inactivated sections mounted over sections of high phosphatase activity (compare his fig. 5 with Pl. 3, fig. 13). He observed nuclear staining in inactivated tissue, however, only when incubation was carried out between pH 7·0 and 8·5; he found no evidence of diffusion at pH 9·2. Because calcium phosphate is highly insoluble at pH 9·2 but much more soluble around pH 7·4 and, therefore, more readily diffusible, Gomori ascribed the false reactions to the migration of the product of phosphatase activity. We purposefully have incubated our sections only at pH 9·4, the optimal level for *in vitro* phosphatase activity, so we have no corresponding data. Martin & Jacoby (1949) observed these diffusion phenomena at pH 9·2. The reason for these differences in pH level at which diffusion may occur is not clear.

The demonstration of phosphatase activity in some nuclei only after prolonged incubation may signify the presence of relatively small amounts of active enzyme so that an extended incubation period is needed to produce sufficient calcium phosphate for visualization. On the other hand, the long sojourn in an alkaline medium may itself activate the enzyme. There are abundant references in the literature to histochemically demonstrable alkaline phosphatase in nuclei, some in tissues in which no foci of intense activity are present (e.g. in tissue culture cells, Chèvremont & Firket, 1949). Newman *et al.* (1950) have classified alkaline phosphatases into three groups. One of these (group III) is the enzyme located specifically in nuclei. It is characterized by a comparatively slower rate of hydrolysis and greater resistance to inhibitors than the cytoplasmic phosphatases. Chemical analyses of isolated nuclei have also revealed the presence of alkaline phosphatase in nuclei (Mirsky, 1947), and Dounce (1950) has found a higher concentration of alkaline phosphatase in hepatic nuclei than in the whole tissue. Thus, in spite of the nuclear affinity for calcium phosphate, even at the pH level considered to be optimal for phosphatase activity (Danielli, 1946), the histochemical demonstration of alkaline phosphatase in nuclei would appear to be valid.

The concentration of alkaline phosphatase into nuclei from acid solutions but not from alkaline solutions of the enzyme suggests that the phosphatase may combine with some specific constituent of nuclei, possibly through a salt-like linkage, which dissociates in acid solutions. Although nucleic acids may still dissociate as acids at pH 5·0, it was found that phosphatase deposition in nuclei was not altered by preliminary removal of all nucleic acid from the sections with 0·1 N-HCl. A combination with nucleic acids is unlikely, furthermore, since they dissociate to a greater degree in alkaline solutions where phosphatase concentration in nuclei does not occur. It would seem more likely that the enzyme could combine with the strongly basic

proteins of the nucleus, but we have no evidence for this at present. That the intrinsic alkaline phosphatase of nuclei is not associated with the desoxyribonucleic acid component of chromosomes has been demonstrated by Brachet & Shaver (1948). Treatment of sections with desoxyribonuclease brought about the disappearance of desoxyribonucleic acid but produced no change in the intensity of the alkaline phosphatase reaction. Mirsky (1947), furthermore, has found that the inherent alkaline phosphatase can be dissociated from the nucleohistone fraction and is bound to the residual tryptophane-containing protein of chromosomes.

In spite of the demonstration of diffusion phenomena in the histochemical technique for alkaline phosphatase, we believe that the method is a reliable one when proper precautions are taken. Short incubation periods reveal the sites of greatest activity. When incubation is prolonged additional sites may appear. These can be interpreted only after proper controls have been used and may indicate the presence of a small amount of enzyme, activated enzyme or, if immediately adjacent to a focus of high activity, stainable material which has diffused and become adsorbed onto tissue elements during incubation.

SUMMARY

The sites of naturally occurring alkaline phosphatase in the guinea-pig liver have been revealed by the use of various substrates and of enzyme activators. Phosphatase activity was found in the nuclei of all hepatic elements and in the cytoplasm of smooth muscle cells, endothelial cells and leucocytes variously associated with the hepatic sinusoids and blood vessels.

An intensification of the histochemical reaction for phosphatase in both nuclei and cytoplasm occurred in sections of guinea-pig liver in areas adjacent to superimposed sections of duodenal mucosa, the epithelium of which contains normally a large amount of phosphatase. This enhanced activity of adjacent hepatic cells resembled that obtained by using chemical activating agents. It is suggested, therefore, that these zones of increased phosphatase activity in the hepatic lobules result, in part, from the activation of intrinsic phosphatase by enzyme activators from the duodenal cells.

Another type of diffusion was encountered when sections of phosphatase-rich duodenum were incubated over sections of liver in which all native phosphatase had first been destroyed by heat. In such preparations a reaction occurred in adjacent liver cells, but it was limited to nuclei immediately contiguous to the superimposed reactive duodenal cells, and it was never observed in cytoplasmic elements of the liver.

The penetration of phosphatase from buffered solutions of the enzyme into hepatic nuclei, in which all intrinsic phosphatase had been destroyed by heat, was most pronounced at pH 5·0, slight at pH 7·0 and absent at pH 9·5. The latter pH is the optimal one for the histochemical demonstration of phosphatase. It appears, therefore, that the reaction observed in enzyme-free nuclei around loci of high phosphatase content is probably not the result of migration or diffusion of the enzyme. It seems more likely that calcium phosphate, the product of the histochemical reaction of phosphatase, is the diffusible substance.

REFERENCES

BOURNE, G. (1943). The distribution of alkaline phosphatase in various tissues. *Quart. J. exp. Physiol.* **32**, 1–19.

BRACHET, J. & SHAVER, J. R. (1948). The effect of nucleases on cytochemical reactions for amino-acids and on staining with acid dyes. *Stain Tech.* **23**, 177–184.

CHÈVREMONT, M. & FIRKET, H. (1949). Phosphatase alcaline dans les cellules cultivées *in vitro*; ses rapports avec la croissance. *Arch. Biol., Paris*, **60**, 441–472.

DANIELLI, J. F. (1946). A critical study of techniques for determining the cytological position of alkaline phosphatase. *J. exp. Biol.* **22**, 110–117.

DEMPSEY, E. W. & DEANE, H. W. (1946). The cytological localization, substrate specificity and pH optima of phosphatases in the duodenum of the mouse. *J. cell. comp. Physiol.* **27**, 159–179.

DEMPSEY, E. W., SINGER, M. & WISLOCKI, G. B. (1950). The increased basophilia of tissue proteins after oxidation with periodic acid. *Stain Tech.* **25**, 73–80.

DOUNCE, A. L. (1950). Enzyme systems of isolated cell nuclei. *Ann. N.Y. Acad. Sci.* **50**, 982–997.

FEIGIN, U., WOLF, A. & KABAT, E. A. (1950). Histochemical studies on tissue enzymes. VI. A difficulty in the histochemical localization of alkaline phosphatase in nuclei. *Amer. J. Path.* **26**, 647–659.

FISCHER, C. J. & GREEP, R. O. (1948). Studies on activation of purified alkaline phosphatase. *Arch. Biochem.* **16**, 199–219.

GOMORI, G. (1939). Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. exp. Biol., N.Y.*, **42**, 23–26.

GOMORI, G. (1941). The distribution of phosphatase in normal organs and tissues. *J. cell. comp. Physiol.* **17**, 71–83.

GOMORI, G. (1950). Sources of error in enzymatic histochemistry. *J. Lab. clin. Med.* **35**, 802–809.

GREEP, R. O., FISCHER, C. J. & MORSE, A. (1947). Histochemical demonstration of alkaline phosphatase in decalcified dental and osseous tissues. *Science*, **105**, 666.

GREEP, R. O., FISCHER, C. J. & MORSE, A. (1948). Alkaline phosphatase in odontogenesis and osteogenesis and its histochemical demonstration after demineralization. *J. Amer. dent. Ass.* **36**, 427–442.

HARD, W. L. & HAWKINS, R. K. (1950). The role of the bile capillaries in the secretion of phosphatase by the rabbit liver. *Anat. Rec.* **106**, 395–412.

LISON, L. (1948). La recherche histochimique des phosphatases. Étude critique. *Bull. Histol. Tech. micr.* **25**, 23–41.

MARTIN, B. F. & JACOBY, F. (1949). Diffusion phenomena complicating the histochemical reaction for alkaline phosphatase. *J. Anat., Lond.*, **83**, 351–363.

MIRSKY, A. E. (1947). Chemical properties of isolated chromosomes. *Cold Spr. Harb. Symp. quant. Biol.* **12**, 143–145.

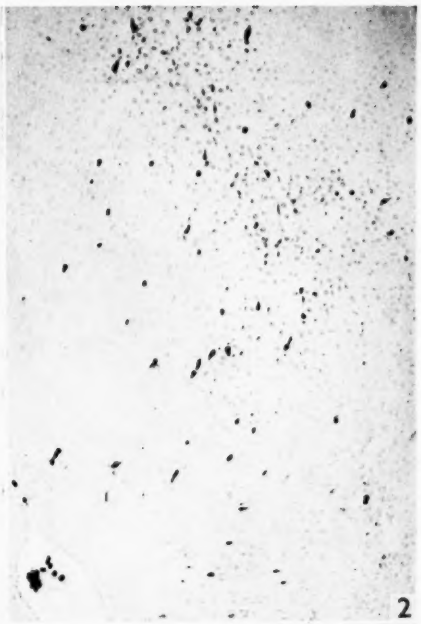
MORSE, A. & GREEP, R. O. (1947). Alkaline glycerophosphatase in the developing teeth of the rat: its localization and activity characteristics as influenced by pH of the substrate and length of incubation time. *Anat. Rec.* **99**, 379–396.

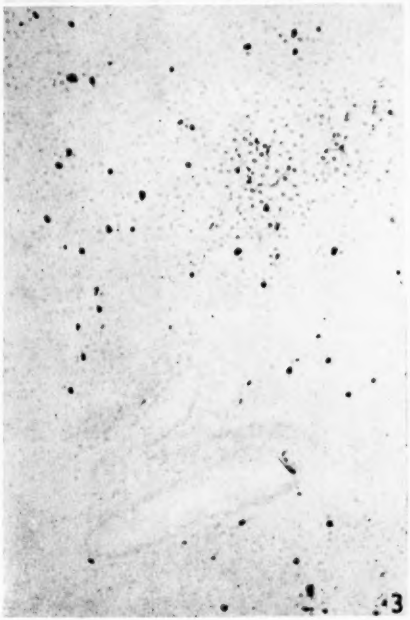
NEWMAN, W., FEIGIN, I., WOLF, A. & KABAT, E. A. (1950). Histochemical studies on tissue enzymes. IV. Distribution of some enzyme systems which liberate phosphate at pH 9·2 as determined with various substrates and inhibitors; demonstration of three groups of enzymes. *Amer. J. Path.* **26**, 257–305.

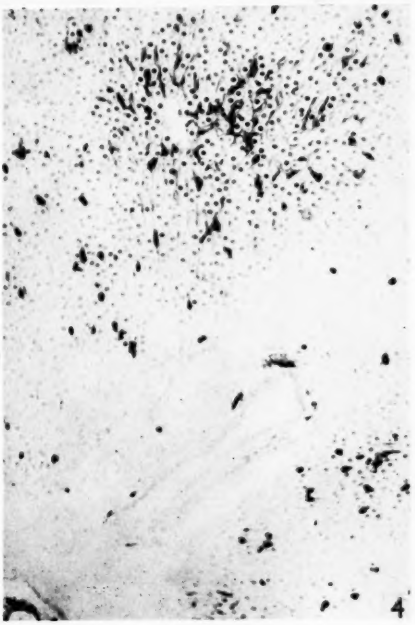
ROCHE, J. & NGUYEN-VAN THOAI (1950). Phosphatase alcaline. *Adv. Enzymol.* **10**, 83–122.

TAKAMATSU, H. (1939). Histologische und biochemische Studien über die Phosphatase. I. Histochemische Untersuchungsmethodik der Phosphatase und deren Verteilung in verschiedenen Organen und Geweben. *Trans. Jap. path. Soc.* **29**, 492–498.

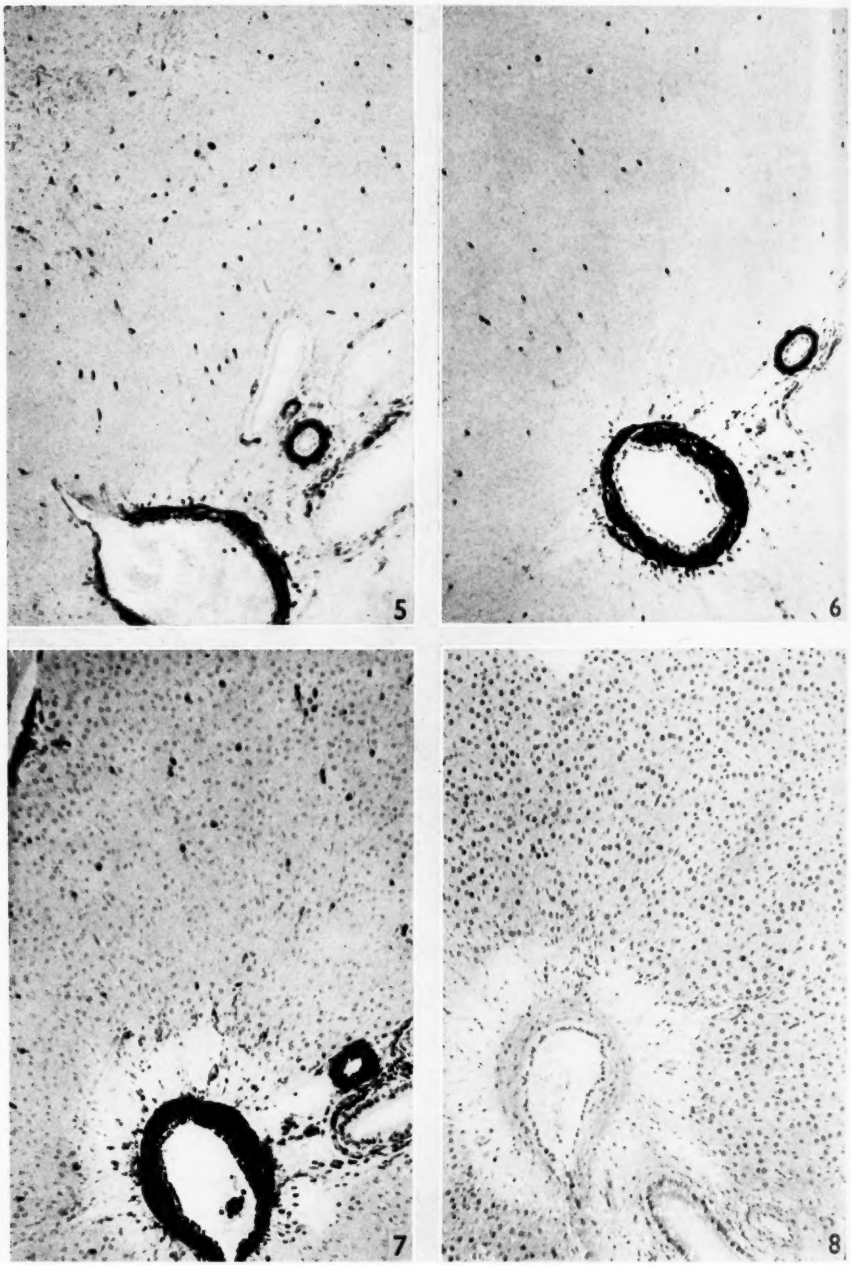
ZORZOLI, A. & STOWELL, R. E. (1947). Comparison of the distribution of an hexosediphosphatase with glycerophosphatase in different tissues. *Anat. Rec.* **97**, 495–505.







LEDUC AND DEMPSEY—ACTIVATION AND DIFFUSION IN ALKALINE PHOSPHATASE REACTION



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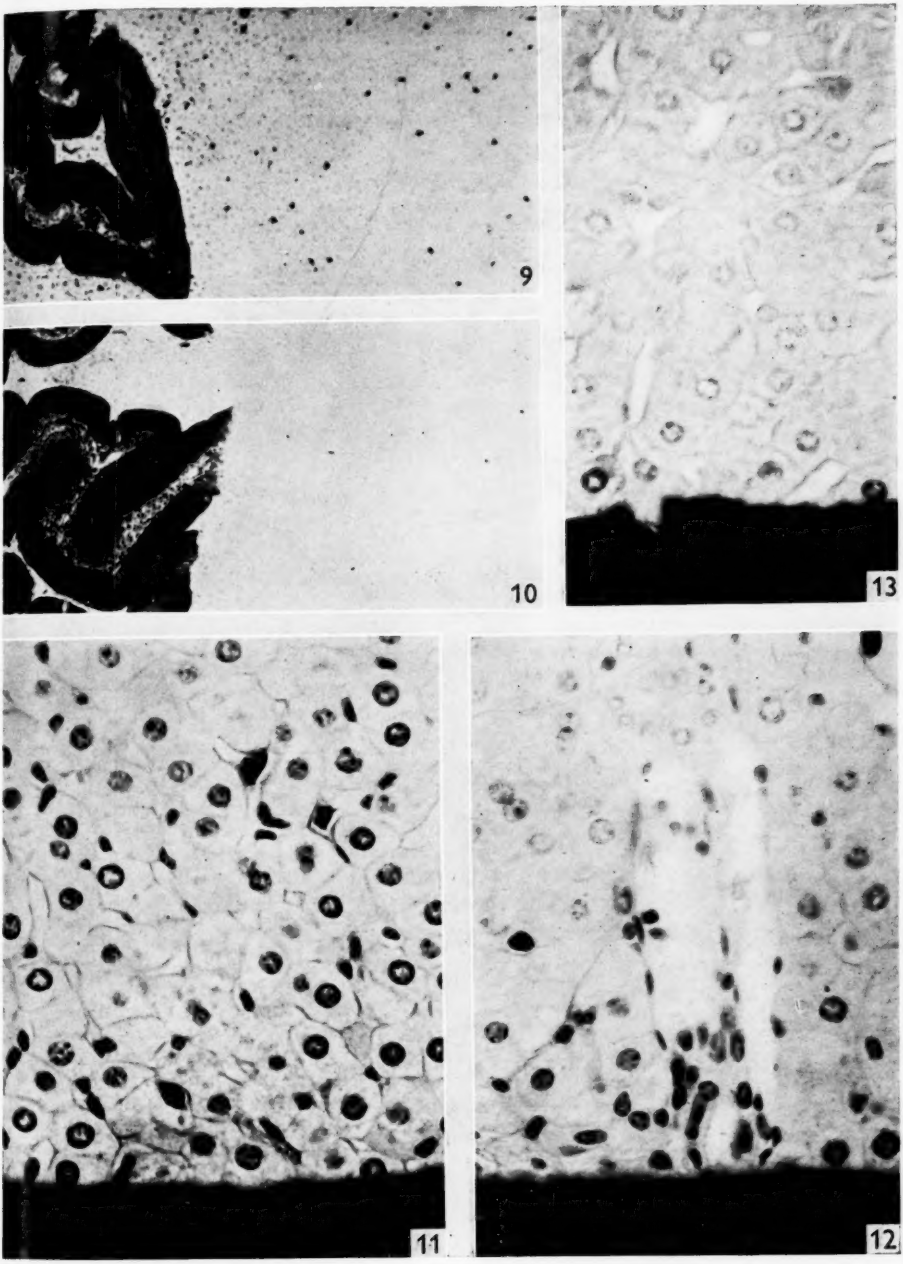

LEDUC AND DEMPSEY—ACTIVATION AND DIFFUSION IN ALKALINE PHOSPHATASE REACTION

EXPLANATION OF PLATES

Figs. 1–8 are from parallel sections of a single block of guinea-pig liver; figs. 9–13, from the same liver with superimposed sections of a rat duodenum. In figs. 1–8 the same portal canal has been included in the photomicrograph. The tissues were fixed in chilled 80% alcohol, embedded in paraffin and sectioned at 5μ. The sites of alkaline phosphatase activity are visualized by brown deposits of cobalt sulphide. No counterstain was used. Figs. 1–10 were taken at ×520.

Plate 1

Fig. 1. Guinea-pig liver incubated in glycerophosphate, pH 9·4, 24 hr. Alkaline phosphatase activity present only in polymorphonuclear leucocytes in the sinusoids.

Fig. 2. The same liver incubated in glycerophosphate substrate plus magnesium chloride, pH 9·4, 24 hr. Phosphatase activity is also exhibited in the nuclei of hepatic cells and of Kupffer cells and in the cells lining the sinusoids in the central zone of the lobule.

Fig. 3. The same liver incubated in glycerophosphate, pH 9·4, 24 hr., after preliminary activation of intrinsic phosphatase activity by trypsin. The nuclei of the centrolobular cells are positive. All portal elements are negative.

Fig. 4. The same liver incubated in glycerophosphate, pH 9·4, 24 hr., with the combined action of the three phosphatase activators, trypsin, sodium barbital and magnesium chloride. The most pronounced intensification of activity in the centrolobular cells occurs in this preparation.

Plate 2

Fig. 5. Guinea-pig liver incubated in fructose diphosphate, pH 9·4, 24 hr. Positive reaction for alkaline phosphatase present in polymorphonuclear blood cells, smooth muscle coats of arteries and nuclei of the connective tissue cells of the portal canal and the hepatic and Kupffer cells in the central zone of the lobule.

Fig. 6. Guinea-pig liver incubated in adenylic acid, pH 9·4, 24 hr. Intense phosphatase activity in polymorphonuclear cells, smooth muscle cells and nuclei of connective tissue cells.

Fig. 7. Guinea-pig liver incubated in nucleic acid, pH 9·4, 24 hr. In addition to sites of intense activity presented in figs. 5 and 6 the nuclei of all cells are positive.

Fig. 8. Guinea-pig liver which was (1) heated in order to destroy intrinsic phosphatase, (2) soaked in a solution of alkaline phosphatase at pH 5·0 and (3) subsequently incubated in glycerophosphate plus magnesium, pH 9·4, 24 hr. Alkaline phosphatase was deposited in all nuclei of this heat-inactivated section.

Plate 3

Fig. 9. Rat duodenum superimposed over guinea-pig liver and incubated in glycerophosphate, pH 9·4, 24 hr. Note broad zone of phosphatase activity in hepatic nuclei in centrolobular areas that are adjacent to the highly active section of duodenum (top of figure).

Fig. 10. Rat duodenum superimposed over guinea-pig liver, in which intrinsic phosphatase had been destroyed by heat, and incubated in glycerophosphate pH 9·4, 24 hr. Faintly positive reaction in hepatic nuclei present only immediately adjacent to section of duodenum. Portal (bottom of figure) and central cells (top of figure) react alike.

Fig. 11. Same preparation as in fig. 9. Broad zone of phosphatase activity in centrolobular hepatic cells adjacent to duodenum. The duodenal epithelium appears solid black in this preparation.

Fig. 12. Same preparation as in figs. 9 and 11 and a field adjacent to that in fig. 11. Narrow zone of activity in portal hepatic cells near section of superimposed duodenum.

Fig. 13. Same preparation as in fig. 10. False positive reaction in heat-inactivated hepatic nuclei adjacent to duodenum.

CYCLICAL CHANGES IN THE UTERINE MUCOSA AND VAGINA OF THE GOAT

By W. J. HAMILTON and R. J. HARRISON
Anatomy Department, Charing Cross Hospital Medical School

INTRODUCTION

Few observations have been made on the changes in the reproductive tract in the goat. Küpfer (1928) has described the macroscopic appearances of the ovaries of twenty Boer and twenty Angora goats from South Africa. One of us (Harrison, 1948) has given an account of the histological changes occurring in the ovary of the goat during the oestrous cycle and in early pregnancy. In that paper brief reference was made to the changes in the reproductive tract; the following work is an addition to the preliminary observations and gives for the first time an account of the cyclical changes occurring in the uterine mucosa and vagina.

Comparison is made with the cyclical changes in the reproductive tracts of other Artiodactyla and the changes are also correlated with those already described in the corpora lutea of the goat (Harrison, 1948). Corner (1921) and Wilson (1926) have described the cyclical changes occurring in the uterus of the sow; Grant (1934), Casida & M'Kenzie (1932) and Cole & Miller (1935) have described those occurring in the sheep; Hammond (1927) and Cole (1930) have given accounts of the histology of the reproductive tract of the cow. Details of the reproductive cycle of these and many other domestic animals have been collected by Asdell (1946). The latter has also summarized the literature dealing with the details of the timing of the reproductive cycle. The length of the cycle in the goat is generally given as about 21 days, and heat lasts about 40 hr. The duration of the cycle varies in different animals and in different breeds, and the duration of heat is subject to similar variation. Ovulation is spontaneous and occurs towards the end of heat.

MATERIALS AND METHODS

The material used in the present investigation consisted of part of that used by one of us (Harrison, 1948) in describing changes in the ovaries during the oestrous cycle. The number of each animal in the following description refers to that given in Table 1 on p. 22 of that paper. It consisted of the genital tracts of seventeen goats killed at certain intervals during the reproductive cycle. The onset of oestrus was, in each instance, determined by the willingness of the female to accept the male. The ages and previous reproductive history were not known and the breeds were indeterminate. Unfortunately, it was not possible to take vaginal smears.

Parts of each genital tract were fixed in Zenker's, Bouin's and in Heidenhain's 'Susa' fixative. Sections were stained with various techniques, but the description which follows is mainly based on sections stained with Harris's or Heidenhain's haematoxylin, and counterstained with eosin, and with Mallory's and various modifications of Masson's stain.

OBSERVATIONS

The uterine horn

The appearances of the mucosa in the uterine horns, both in the fused and the unfused portions are essentially similar. In both parts of the horn the lumen is stellate in shape (Pl. 1, figs. 1, 2). The shape of the lumen of each horn at different levels shows considerable variation in any animal and there does not appear to be a definite pattern.

Oestrus

The mucosa of the uterine horns of goats 14 and 18, which were killed on the first day of the oestrous cycle, will be described first; both animals were in heat but had not yet ovulated.

The epithelium is tall, columnar (50–60μ in height), non-ciliated, with the nuclei situated at different levels in the cells so as to suggest pseudo-stratification. Two types of nuclei can be seen, one fusiform and intensely stained, the other oval, vesicular and lightly stained. At intervals along the epithelium, superficial to the basement membrane, epithelial cells can be seen which contain strongly basophil material arranged in closely packed rods, quite distinct from the nucleus.

The stroma is indistinctly divided into a denser, narrow zone deep to the epithelium and a looser broad outer zone. In the latter, there are patches of oedema. In the superficial zone there are localized areas of lymphocytic infiltration, and lymphocytes are occasionally found in the epithelium also. The stromal cells are oval or polyhedral; there are occasional strands of collagenous tissue near the periphery. Large pear-shaped or oval cells containing a yellow pigment are widely distributed in the deep zone of the stroma.

Irregularly placed in the mucosa there are caruncular areas with a fairly well-defined contour. These contain closely packed masses of stromal cells which are separated into irregular aggregations by whorls of fibroblasts.

The glands are uniformly distributed throughout the mucosa, except in the caruncular region where they are absent. They pursue a sinuous course towards the muscle coat and their basal parts become coiled. The cells of the glands are columnar and are taller towards the lumen of the uterus than they are in the basal area. The lumina of the ducts and of the more superficial parts of the glands are larger than in the deeper parts, where the lumen may be very small. The same two types of nuclei referred to above, one intensely stained, the other palely stained, are found in the glandular epithelium, but the differences are not so marked as in the uterine epithelium. The presence of a large number of blood vessels in the mucosa is not a striking feature at this stage.

Goat 28 was in oestrus but had recently ovulated. The appearances of the epithelium and mucosa are essentially similar to those described above.

Goats 29 and 13 were killed on the second day of the cycle between 6 and 18 hr. after ovulation. The appearance of the mucosa in the two animals is so alike that they may be considered together (Pl. 2, fig. 6). The epithelium is lower (36μ) but there is still evidence of pseudo-stratification. The two types of nucleus, although discernible, cannot be differentiated easily. All the nuclei are now oval.

Aggregations of basophil material in the epithelial cells can be found in most sections. Lymphocytes can also be seen in the epithelium.

The stroma is now more oedematous than in the preceding specimens; there is little change in the caruncles; lymphocytes are present either singly or in patches. There is evidence of increased vascularity of the mucosa in both animals. The glands are more numerous and slightly more dilated, but there is no active secretion.

Appearances during first week after oestrus

Goats 26 and 31 were killed on the 3rd day of the cycle, and goat 25 was killed on the 6th day. The corpus luteum was much better developed in goat 25 than in goats 26 or 31.

The epithelium of goats 26 and 31 is a little lower (30μ) than in goats 29 and 13, but the general characters are similar. The glands are more numerous and slightly more dilated. The vascularization of the stroma is well advanced and there is also marked oedema. The epithelium of goat 25 has not changed so much as in the preceding specimens, and is reminiscent of the epithelium at oestrus. However, despite the fact that the stroma is less oedematous, the glands show more advanced changes than any so far described. In almost every gland there is evidence of impending secretory activity. The epithelial cells are large, the nuclei lie at the base of the cell and there is a larger lumen, sometimes containing mucus. Therefore it appears that although the corpus luteum is functioning, the changes in the mucosa are not always identical.

Appearances in the second week of the cycle

Goats 36 and 15 were killed on the 9th day, goat 30 was killed on the 12th day and goat 32 on the 13th day (Pl. 2, figs. 7, 8). The corpus luteum is actively secreting at this stage and reaches its maximum diameter on the 12th day.

The epithelium in the earlier stages of this period is still pseudo-stratified, but by the 13th day it is becoming columnar. The nuclei are found at different levels in the cells, but are mainly in the middle third. Vacuoles are present in the luminal part of the cell. The glands are large and dilated and secretory products are present in them. The mucosa is oedematous and there are patches of hyaline degeneration.

Appearances in the third week of the cycle

The epithelium of goat 27, killed on the 16th day, is regular and the cells are columnar (Pl. 2, fig. 9). The nuclei are more regularly arranged and are mainly basal in each cell. The stroma is less oedematous and all the glands are less active. The compact zone of the mucosa is more definite and wider than in previous specimens. By the 17th day, goat 33, the epithelium is still columnar and the nuclei of all the epithelial cells are situated in the central or basal part of each cell. The glands are still active, and the nuclei of their epithelium are basal in position. The stroma about the glands in particular is oedematous and loosely arranged.

By the 19th and 20th days of the cycle, goats 35 and 34, the epithelium is columnar and regular and the nuclei are basal in position (Pl. 2, fig. 10). The stroma is dense and has a firm fibrous appearance. A few lymphocytes are scattered in the

superficial part of the mucosa. The glands are inactive, with no secretion in the lumen, and the nuclei of their cells are all regularly placed and lie in the basal part of the cell.

Cervix

Oestrus

The lumen of the cervix is divided by a number of longitudinal folds (25–30 in number) which project into it, but do not meet in the centre (Pl. 1, fig. 3). The tips of adjacent folds are separated by nearly 1 mm. The folds at the opening into the vagina are shorter and simpler than those higher up; they are on the average 0·75–1 mm. in length and 0·2 mm. thick, but the measurements vary at different times in the cycle. About 1 cm. above the opening into the vagina the folds are larger and more complicated, subdividing at the tips to give a number of secondary folds, each fold having a foliate appearance in cross-section. The folds at this level are 1–3 mm. long and between 0·5 and 1 mm. thick. No muscle fibres pass into the folds. The lumen contains strands of an amorphous material which is probably mucoid in nature, some granular material and a few red blood cells.

At the base of the cleft between each main fold, short branched recesses having the form of dilated glands penetrate the stroma for about 1 mm. (Pl. 1, fig. 5). The recesses between the folds tend to be arranged in groups; between each group there is intervening stromal tissue which extends into each fold. The recesses extend towards the inner layer of circular muscle, but do not penetrate the muscle coat. The latter contains considerable quantities of fibrous tissue, which increases in amount towards the lumen.

The stroma of the basal mucosa and of the folds is similar in appearance. It consists of a somewhat loosely arranged areolar tissue containing fibroblasts, collagenous fibres, small round cells (lymphocytes), larger polyhedral cells (stromal cells) and numerous small blood vessels. The lymphocytes are found mainly at the bases of the folds in relation to the blood vessels entering each fold. In certain places, namely in the basal zone of the mucosa and at the tips of the folds, there are areas where the stroma is oedematous. The blood supply to the folds is extensive, vessels entering the base of each fold and leading to a marked vascular plexus within the fold extending to its tip.

The epithelium covering the tips of the folds is tall columnar. The cells are 45μ high, 6μ across at the top and 3–4μ across at the base. The cells are of two distinct types; a ciliated columnar cell with a palely staining nucleus and a non-ciliated goblet cell with a darker staining nucleus. The two types of cell alternate fairly regularly. The goblet cells are not actively secreting, but occasional cells can be seen with small clear droplets in their superficial part. The nuclei are usually placed in the middle third of the cells.

The epithelium lining the recesses between the folds is also tall columnar in type, but is only 30μ in height. There are a great many more goblet cells in the recesses, the ratio being approximately three goblet cells to one ciliated columnar cell. The goblet cells in the recesses are more active than those of the folds. Mucigen granules are frequent and trichrome staining shows large blue droplets in the outer cytoplasm. The nuclei are situated mainly in the basal third of the epithelial cells lining the recesses.

At intervals along the epithelium, particularly at the tips and sides of the folds, small groups or islands of polyhedral cells are present lying between the epithelium proper and the basement membrane (Pl. 1, fig. 4). Mitoses are frequently seen and the cytoplasm of these cells is generally darker, due to numbers of eosinophil granules. It is probable that these cells represent a discontinuous germinal layer which is proliferating in response to oestrogenic stimulation. Groups of such cells are also present at the bases of the short secondary folds which are forming in the main fold. In some places a new recess can be seen in cross-section, and the polyhedral darker-staining cells are arranged in several layers about the epithelial cells lining the recess.

No true glands can be seen in the mucosa of the cervix except at its upper end where it joins with that of the short body of the uterus. The appearances of these glands are similar to those of the uterine horn at the same stage of the oestrous cycle. Some of these glands open into the uppermost of the recesses of the cervix.

The first week of the cycle

Goats 26, 31 and 25 were all killed during the first week of the cycle. The folds have elongated and thickened so that they almost completely fill the lumen. Some of them are 3 to 4 mm. in length and between 0·25 and 0·75 mm. thick. There is considerable formation of secondary folds and there is evidence of new folds being formed. The spaces between the folds contain an increased amount of mucoid material. The stromal cells have considerably enlarged, their nuclei are round or oval and stain palely. The blood vessels are dilated, each fold is now very vascular with areas of oedema near the tip. Numbers of lymphocytes are also present evenly distributed in the stromal tissue.

The epithelial cells covering the folds are not so tall as at oestrus, but they are considerably broader. They are some 25–30μ in height and up to 15μ across. The non-ciliated cells are palely staining and considerably distended with secretion. The nuclei of the ciliated cells, whose cytoplasm is more heavily stained, lie in the basal third of the cell. The alternation of ciliated and goblet cells can be clearly seen on this account.

The recesses between each of the folds are now more prominent and very much resemble large dilated glands. There is, however, still the same alternation of ciliated and goblet cells in their walls. The goblet cells are very much enlarged and their cytoplasm is clear and translucent. There is as yet little free secretion in these recesses. There are still areas of epithelial proliferation, particularly at the bases of the recesses. There are also small areas close to these recesses which show marked lymphocytic infiltration.

The second week of the cycle

In goat 36 there is little change from the preceding specimens, whereas goat 15, also killed on the 9th day of the cycle, shows the maximal changes to be seen in this series. The folds have reached their greatest degree of enlargement and of secondary folding. There is considerable mucus in the lumen. The epithelium is 30μ in height. The two types of cell are still discernible, the nuclei are in many cases in the middle or superficial third of the cell. Few of the cells contain secretory droplets. The recesses

are now very marked, some with a diameter of more than 0·25 mm., and they have the appearance of a number of much dilated and convoluted tubules. Several such tubules open into the spaces between two main folds in any particular section. The cells lining the recesses have an appearance similar to those covering the folds. There is much mucus in the recesses.

The stroma is still oedematous, but is more compressed than in the previous specimens, apparently due to the great increase in the number and size of the tubular recesses. The whole mucosa is now very vascular and the vessels are engorged. Lymphocytes are present, either singly or in small masses.

The epithelium covering the folds of goats 30 and 32, killed on the 12th and 13th day of the cycle respectively, is lower, being between 20 and 24μ in height. Less mucus is being secreted, and the epithelium of the recesses is not so active as in the earlier part of this period.

The third week of the cycle

The folds have diminished in size in goats 27, 33, 35 and 34, killed respectively on the 16th, 17th, 19th and 20th day of the cycle, and the epithelium has decreased, being 12–18μ in height. The nuclei now occupy either the central or peripheral part of the cell, and it is difficult to find an area which is pseudo-stratified. Some of the secretory cells in the recesses are still active and a little mucus is present (Pl. 1, fig. 5). At the tip of one of the folds in goat 33, there is a small cyst-like gland, some 150μ in diameter, with flattened cells lining it, and which is filled with mucus.

The epithelium of goat 24, which was killed on the last day of the cycle, is much like that of goats 14 and 18, killed on the 1st day of the cycle. The epithelium is not so tall, however, being only 18–20μ in height. The nuclei are placed at the base of each cell. The epithelium at the base of each recess is proliferating; the cells are several layers thick and there are numerous mitotic figures. The nuclei of these cells are placed in the central part of the cell. There is no evidence of secretion.

Vagina

At oestrus (goat 14) the wall of the vagina is thrown into a number of small well-marked longitudinal folds which are most pronounced at this time. The stratified squamous epithelium is not of uniform thickness throughout, but shows at fairly regular intervals definite papilliform projections into the underlying stroma. The epithelium in the areas between the projections consists of about ten to fifteen cell layers (Pl. 2, fig. 11). The basal cells of the epithelium are cuboidal or columnar; in the papilliform projections, however, many of the cells are polyhedral. The intermediate layers consist of elongated, flattened cells with their long axes parallel to the basement membrane. Most of these cells are granular, densely staining and eosinophil. The more superficial cells are still elongated, but are swollen and their cytoplasm is more basophil. Numerous cells are considerably swollen and have an almost hyaline or neutrophil appearance of the cytoplasm. Many of the most superficial cells are losing their nuclei and stain brightly with picric acid or orange G. Occasional small vacuoles may be seen in the cytoplasm of these cells. Active desquamation is occurring and, as a result, flattened non-nucleated squamous cells and also densely staining nucleated cells, are present in the vagina.

In most areas of the vagina of goat 18 desquamation on the surfaces of the folds is already complete, leaving behind the swollen hyaline cells; in the recesses cellular desquamation is still occurring.

By the 2nd day of the cycle, goats 28, 13 and 29, the epithelium is thinner, being five to eight cells thick, due to the complete desquamation of the superficial layers (Pl. 2, fig. 12). The majority of the remaining cells of the vaginal epithelium are polyhedral. The surface of the epithelium is irregular, owing to the desquamation of polyhedral cells. A clear-cut boundary between the bas layer and the stroma is lost in many places. A few lymphocytes are present hin and beneath the epithelium.

The epithelium of the vagina of goats 26 and 31 at the 3rd ay is thicker. There is some flattening of the superficial layers of cells, but no kera ization. There is still no distinct boundary between the epithelium and the subj nt tissue. There are now increased numbers of lymphocytes, both in the epithelium nd in the underlying tissue. The latter is now markedly oedematous.

By the period covering the 6th to the 9th day (goats 25, 36 nd 15) the epithelium has become further thinned over the vaginal folds, which are not as marked as at oestrus. There is more marked lymphocytic invasion of the epithelium, and there are isolated aggregations of lymphocytes in the subepithelial region. The latter has lost its cellular appearance and is more fibrous and oedematous than in the previous specimens.

By the 12th day, goats 30 and 32, however, the epithelium is much reduced, being three or four cells thick. The appearances now resemble those of a transitional epithelium, the basal cells are cuboidal, the intermediate cells are larger, polyhedral or pear-shaped and extend as far as a single-celled surface layer, which is composed of flattened non-keratinized cells (Pl. 2, fig. 13). The base of the epithelium is supported by numbers of elongated connective tissue cells compressed against the basement membrane. Only a few lymphocytes are present.

A few days later, on the 16th and 17th days of the cycle, goats 27 and 33, the epithelium becomes thicker. The line of demarcation at the basal area is not clear-cut, and there is some lymphocytic infiltration. The epithelium has lost its transitional character, except in a few places in the superficial zones. The cytoplasm of all the epithelial cells has a hyaline appearance. There is an increase in the collagenous material in the subepithelial zone and the nuclei of the connective tissue cells are large and swollen.

The vagina of goats 35 and 34, killed respectively on the 19th and 20th days of the cycle, has a thinner epithelium, better demarcated from the underlying tissue, and with several layers of flattened superficial cells (Pl. 2, fig. 14). There is not yet any keratinization of these cells. The underlying tissue has lost its cellular appearance and there are very few lymphocytes.

COMMENTS

The cyclical changes in the reproductive tract are closely associated with the changes which occur in the ovary and the corpus luteum (Harrison, 1948). The central cavity of the corpus luteum is obliterated by the 7th day and the granulosa cells are then fully lutealized; the corpus luteum reaches its maximum diameter by the 12th day.

After the 12th day the luteal cells steadily retrogress until the beginning of the next cycle. On the other hand, the changes which occur in the genital tract are at certain times relatively abrupt in onset and short in duration. On the 20th day of the cycle there is little evidence of cornification in the vagina, yet on the 1st day of the cycle, cornification is very marked. It is well advanced on the 2nd day, but on the 3rd day there is little evidence of desquamation. In the sheep also Grant (1934) finds that heat comes on rapidly but, in contradistinction to the goat, disappears slowly.

Although the changes in the genital tract of the goat are distinct for each period of the cycle, they are not so marked as those found in many rodents and carnivores.

The most pronounced changes occur in the vaginal epithelium during the first few days of the cycle and are more marked than in any other part of the genital tract. The sudden appearance of cornification and the rapid desquamation would indicate an intense heat of short duration. The relatively inactive state of the vaginal epithelium during the remainder of the cycle is interrupted around the 12th day by its transformation into a transitional epithelium. This is undoubtedly due to the activity of the corpus luteum at this time (Harrison, 1948).

The changes occurring in the uterine horn and cervix do not present such a striking picture as those in the vagina.

The epithelium and the glands show maximal secretory activity from the 9th to 13th day of the cycle. Thereafter there is a steady decline in activity which corresponds closely to the retrogressive changes in the corpus luteum.

The changes in the uterus are essentially similar to those described by Corner (1921) for the sow, and can be closely correlated with those in the cow (Cole, 1930).

SUMMARY

1. The histological changes in the reproductive tract of the goat are described.

2. The most marked changes are found in the vaginal epithelium during the first 2 days and on the 9th–12th day of the cycle.

3. The changes indicate that oestrus is intense and of short duration; that the corpus luteum exerts its maximal effect from the 9th to 12th day, and that there is subsequent steady retrogression.

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REFERENCES

Asdell, S. A. (1946). *Patterns of Mammalian Reproduction.* New York: Comstock.

Casida, L. E. & M'Kenzie, F. F. (1932). The oestrous cycle of the ewe; histology of the genital tract. *Bull. Univ. Mo. Agric. Exp. Sta. Res.* no. **170**, 1–28.

Cole, H. H. (1930). A study of the mucosa of the genital tract of the cow, with special reference to the cyclic changes. *Amer. J. Anat.* **46**, 261–301.

Cole, H. H. & Miller, R. F. (1935). Changes in the reproductive organs of the ewe with some data bearing on their control. *Amer. J. Anat.* **57**, 39–97.

Corner, G. W. (1921). Cyclic changes in the ovaries and uterus of the sow and their relation to the mechanism of implantation. *Contr. Embryol. Carneg. Instn*, **13**, 119–146.

Grant, F. (1934). Studies on the physiology of reproduction in the ewe. Part II. Changes in the vagina and cervix. *Trans. Roy. Soc. Edinb.* **58**, 16–35.

HAMMOND, J. (1927). *The Physiology of Reproduction in the Cow.* Cambridge University Press.

HARRISON, R. J. (1948). The changes occurring in the ovary of the goat during the oestrous cycle and in early pregnancy. *J. Anat., Lond.*, **82**, 21–48.

KÜPFER, M. (1928). The sexual cycle of female domesticated animals. *13th and 14th Reports of the Dir. of Vet. Educ. and Res. Dep. of Agric., Pretoria, Union of S.A.* Pt II, 1209–1270 and suppl.

WILSON, K. M. (1926). Histological changes in the vaginal mucosa of the sow in relation to the oestrous cycle. *Amer. J. Anat.* **37**, 417–431.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Low-power photograph of a transverse section of the uterine horn of goat 31, killed on the 3rd day of the cycle. ×3·5.

Fig. 2. Low-power photograph of a transverse section of the fused uterine horns of goat 31. ×3·5.

Fig. 3. Low-power photograph of a transverse section of the cervix of goat 31. ×3·5.

Fig. 4. Photomicrograph of a section through one of the cervical folds of goat 14. Note small islands of polyhedral cells lying deep to the epithelium. ×200.

Fig. 5. Low-power photograph of the bases of the cervical folds and the cervical recesses of goat 35. ×66.

PLATE 2

Fig. 6. Photomicrograph of the mucosa of the uterine horn of goat 29, killed on the 2nd day of the cycle. ×160.

Fig. 7. Photomicrograph of the mucosa of the uterine horn of goat 15, killed on the 9th day of the cycle. ×160.

Fig. 8. Photomicrograph of the mucosa of the uterine horn of goat 30, killed on the 12th day of the cycle. ×160.

Fig. 9. Photomicrograph of the mucosa of the uterine horn of goat 27, killed on the 16th day of the cycle. ×160.

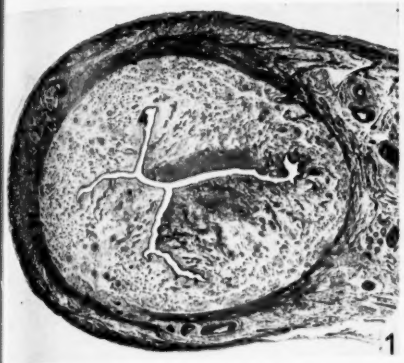
Fig. 10. Photomicrograph of the mucosa of the uterine horn of goat 34, killed on the 20th day of the cycle. ×160.

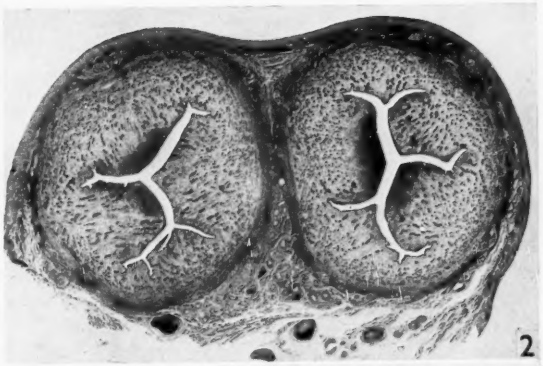
Fig. 11. Photomicrograph of the vaginal epithelium of goat 14, killed on the 1st day of the cycle. ×325.

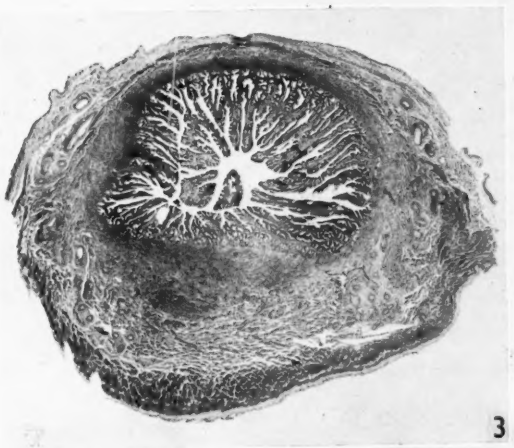
Fig. 12. Photomicrograph of the vaginal epithelium of goat 29, killed on the 2nd day of the cycle. ×325.

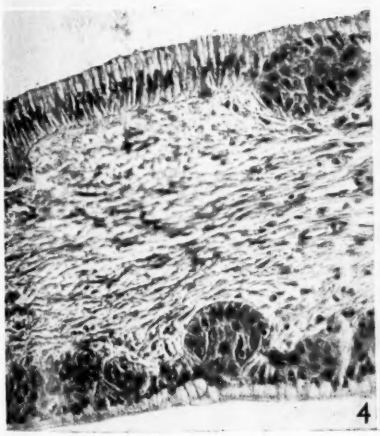
Fig. 13. Photomicrograph of the vaginal epithelium of goat 30, killed on the 12th day of the cycle. ×325.

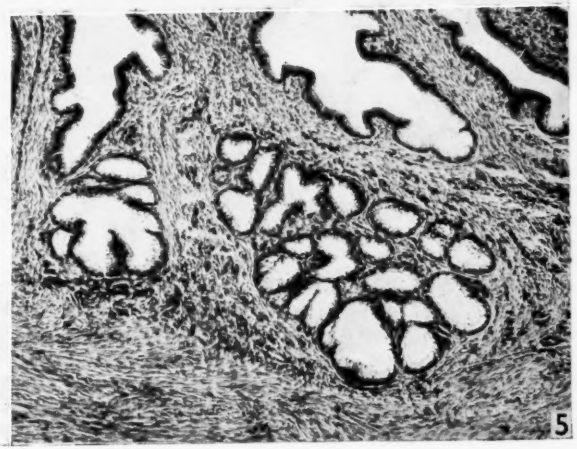
Fig. 14. Photomicrograph of the vaginal epithelium of goat 35, killed on the 19th day of the cycle. ×325.



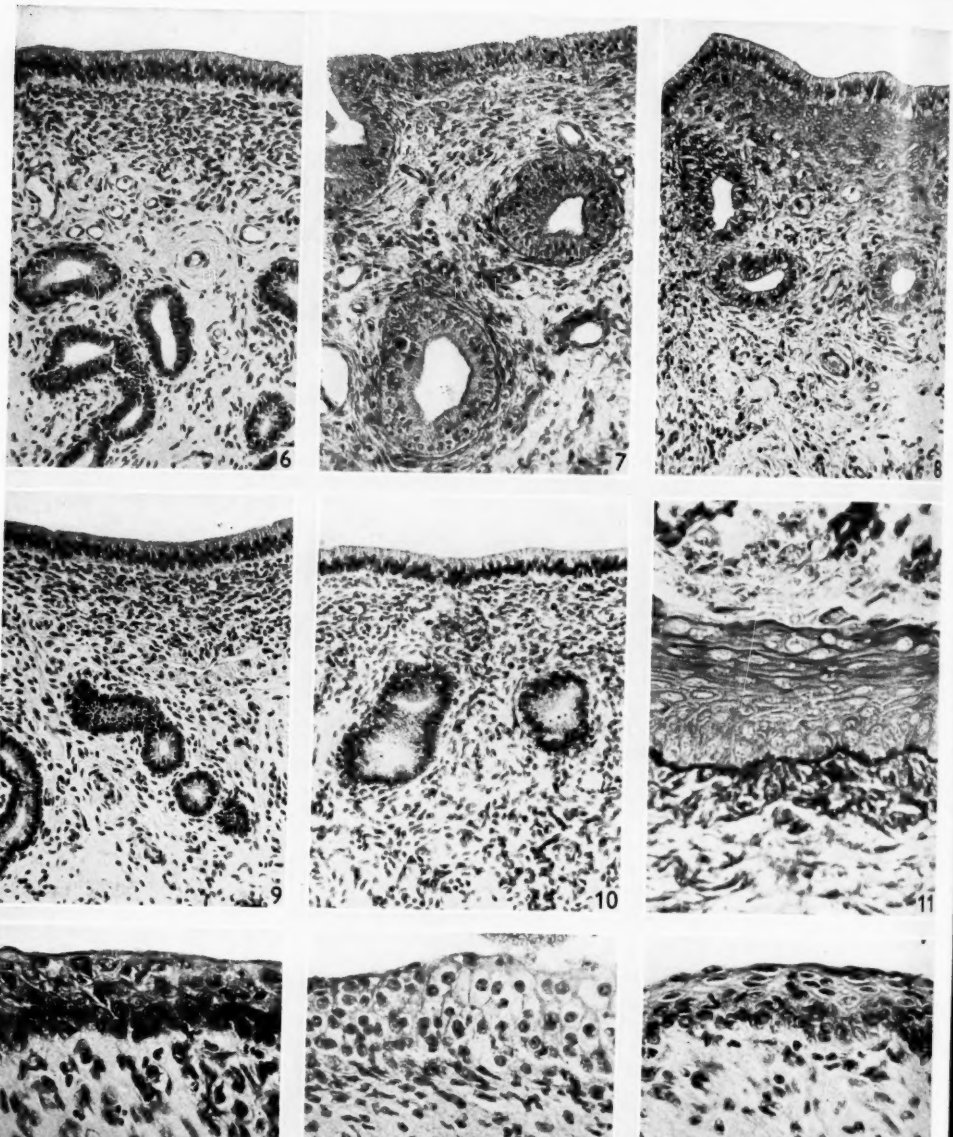








HAMILTON AND HARRISON—CYCLICAL CHANGES IN THE UTERINE MUCOSA AND VAGINA OF THE GOAT



HAMILTON AND HARRISON—CYCLICAL CHANGES IN THE UTERINE MUCOSA AND VAGINA OF THE GOAT

THE PROPORTION OF OVARIAN FOLLICLES IN DIFFERENT STAGES OF DEVELOPMENT IN RATS AND MONKEYS

By S. H. GREEN, A. M. MANDL and S. ZUCKERMAN
Department of Anatomy, University of Birmingham

The active mammalian ovary contains follicles in all stages of development. Oocytes surrounded by an incomplete cellular cover or by a single layer of cells generally make up 90% or more of the total present, while the frequency with which larger follicles occur decreases inversely and exponentially with size. Desaive (1940, 1947, 1948), who has made an intensive study of the ovary of the rabbit, classifies ovarian follicles in nine stages of development, which he defines in terms of size and histological characteristics. He states that the proportion of follicles, out of the total present in the ovary, which is to be found in each of the nine stages of development does not vary, and largely on the basis of this belief he has derived certain precise laws of follicular growth, of which the following three are representative:

(1) Each follicle, considered in isolation, increases in volume according to the exponential law

$$v_t = v_p e^{kt},$$

where v is the volume of the follicle, considered to be a sphere, t is the interval of time in which a follicle increases in size from v_p to v_t and k is a constant.

(2) The length of time θ in which a follicle remains in each stage of development is constant, and is equal to the interval between successive waves of growth of the youngest oocytes.

(3) In the rabbit $\theta = 32{\cdot}8$ hr., and the complete evolution of a follicle takes 11 days ($32{\cdot}8$ hr. $\times 8$).

In view of the importance of these generalizations to an understanding of ovarian function, it was of interest to see whether the proportion of follicles in different stages of development is also constant in the rat and monkey.

MATERIAL AND METHODS

Rat

Total oocyte counts (including both normal and atretic oocytes) of thirty-six laboratory-bred albino rats, ranging in age from 60 to 262 days, were available for analysis. The techniques followed in counting, and the basic data obtained, are given in Mandl & Zuckerman (1950, 1951*a*). Follicles were classified in the following stages:

(1) Primordial ova with incomplete cellular cover, or with complete single layer of cells.
(2) Ova completely surrounded by two layers of granulosa cells.
(3) Ova completely surrounded by three layers of granulosa cells.
(4) Ova completely surrounded by four or more layers of granulosa cells.
(5) Follicles with an antrum.

The data were grouped according to the stages of the oestrous cycle at which the animals were killed (early dioestrus, dioestrus, late dioestrus, early oestrus, oestrus, late oestrus).

The number of oocytes falls off with age, and all oocyte counts were therefore standardized for an age of 100 days, using the regression

$$\text{Log } y = 4{\cdot}561 - 0{\cdot}476 \log x \text{ (Mandl \& Zuckerman, 1951}b\text{),}$$

where y = number of oocytes and x = age in days.

Monkey

Oocyte counts were available for twelve mature rhesus monkeys (*Macaca mulatta*) (Green & Zuckerman, 1951). Because of the lack of sufficient data, the regression of number of oocytes on age could not be determined as accurately for the monkey as for the rat. The data were standardized for an age of 40 months using the linear equation:

$$y = 168332 - 955x,$$

where y = number of oocytes, and x = age in months.

Follicles were classified in the following six groups:

(1) Primordial ova surrounded either by an incomplete or by a complete single layer of cells.

(2) Ova surrounded by two layers of granulosa cells.

(3) Ova surrounded by three layers of granulosa cells.

(4) Ova surrounded by four to six layers of granulosa cells.

(5) Ova surrounded by six or more layers of granulosa cells.

(6) Follicles with an antrum.

Statistical analysis

The χ^2 analysis was used as a test of homogeneity to determine whether the differences between ovaries in the numbers of follicles in various stages of development were significant. The data for each of the six phases of the cycle of the rat were analysed separately. A test of homogeneity was also applied to the data for the right and left ovaries. In addition to a test of homogeneity for all the figures taken together, the data for the monkey were analysed to discover whether the proportions of different sized follicles were the same in the two ovaries, and whether they were constant for the first, middle and last thirds of the menstrual cycle (days 1–9, 10–18, 19–27).

RESULTS

Rats

The mean standardized numbers and the percentages of oocytes at different stages of growth for the six phases of the oestrous cycle are given in Table 1.

χ^2 analyses for the six phases showed that the numbers of follicles in different stages of growth differed in a highly significant way as between stages of the cycle ($P < 0{\cdot}001$). They also showed that in this respect there was a real discrepancy between the right and left ovaries of the same animal.

Monkeys

The basic data for the monkey are given in Table 2. χ^2 analyses showed that, as in the rat, there were highly significant differences ($P < 0{\cdot}001$) between the numbers of follicles in different stages of growth, whichever way the data were grouped for analysis ((1) all data considered together; (2) data partitioned between the first, middle and last thirds of the cycle; (3) between right and left ovaries).

Table 1. *Mean number of oocytes in both ovaries, standardized for age of 100 days, at different phases of the oestrous cycle of the rat.*

(The mean percentages of oocytes at different stages of development in black.)

State of oestrous cycle	No. of oocytes					Total
	Group 1	Group 2	Group 3	Group 4	Group 5	
Early dioestrus	3563	70	81	113	84	3912
	90·1	**2·0**	**2·3**	**3·2**	**2·4**	
Dioestrus	3901	59	67	94	69	4190
	93·1	**1·4**	**1·6**	**2·3**	**1·7**	
Late dioestrus	4163	67	50	86	63	4429
	93·9	**1·5**	**1·1**	**2·0**	**1·5**	
Early oestrus	4762	95	75	77	87	5095
	93·5	**1·8**	**1·5**	**1·6**	**1·7**	
Oestrus	4800	64	65	113	71	5113
	93·5	**1·3**	**1·3**	**2·4**	**1·5**	
Late oestrus	4573	63	63	124	68	4891
	93·2	**1·3**	**1·3**	**2·7**	**1·5**	

Table 2. *The number of oocytes in rhesus monkeys, standardized for an age of 40 months, at different times of the menstrual cycle.*

(The percentages of oocytes at different stages of development in black.)

Animal no.	No. of oocytes						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Total
				Days 1–9			
76 (L)	163,671	2,651	779	697	69	45	167,912
	97·5	**1·6**	**0·5**	**0·4**	**0·0**	**0·0**	
58	85,326	7,798	3,333	2,151	137	363	99,108
	86·1	**7·9**	**3·4**	**2·2**	**0·1**	**0·4**	
382	196,936	8,547	4,184	4,470	159	71	214,368
	91·9	**4·0**	**2·0**	**2·1**	**0·1**	**0·0**	
56	115,920	4,074	1,596	821	77	190	122,678
	94·5	**3·3**	**1·3**	**0·7**	**0·1**	**0·2**	
27	179,982	6,187	3,936	2,739	172	398	193,415
	93·1	**3·2**	**2·0**	**1·4**	**0·1**	**0·2**	
Mean	148,367	5,851	2,766	2,176	123	213	159,496
	92·6	**4·0**	**1·8**	**1·4**	**0·1**	**0·2**	
				Days 10–18			
76 (R)	176,976	2,963	1,463	1,359	82	60	182,904
	96·8	**1·6**	**0·8**	**0·7**	**0·0**	**0·0**	
57	137,608	12,199	4,115	3,324	126	295	157,666
	87·3	**7·7**	**2·6**	**2·1**	**0·1**	**0·2**	
73	69,897	6,282	2,848	4,413	327	308	84,076
	83·1	**7·5**	**3·4**	**5·2**	**0·4**	**0·4**	
600	84,866	1,534	788	469	36	200	87,893
	96·6	**1·7**	**0·9**	**0·5**	**0·0**	**0·2**	
Mean	117,337	5,745	2,304	2,391	143	216	128,135
	90·9	**4·6**	**1·9**	**2·2**	**0·1**	**0·2**	
				Days 19–27			
614	16,267	483	188	250	25	79	17,292
	94·1	**2·8**	**1·1**	**1·4**	**0·1**	**0·5**	
64	158,983	6,013	2,677	1,532	260	156	169,620
	93·7	**3·5**	**1·6**	**0·9**	**0·2**	**0·1**	
602	142,982	7,555	2,262	398	51	315	153,562
	93·1	**4·9**	**1·5**	**0·3**	**0·0**	**0·2**	
541	95,647	6,769	5,024	2,369	376	540	11,0724
	86·4	**6·1**	**4·5**	**2·1**	**0·3**	**0·5**	
Mean	103,470	5,205	2,538	1,137	178	273	112,800
	91·8	**4·3**	**2·2**	**1·2**	**0·2**	**0·3**	

DISCUSSION

The process of follicular growth from the stage of the primordial oocyte to the mature follicle is something which we infer from the observation that all stages between the primordial oocyte and the mature follicle are to be seen in histological preparations of the ovary. The only part of the process that has actually been observed is the final stage of follicular swelling and the rupture of the follicle. Desaive's studies of the development of the ovarian follicle represent the most ambitious attempt yet made to 'set in motion' the static phases of follicular growth that are to be seen in one, or in any series of sections of an ovary, in so far as he has tried to infer from the numerical relations of follicles of different size the actual time taken by the follicle in its development. He starts from the observation that the ovary contains, as already observed, very large numbers of primordial, and a very small number of mature follicles. In his view the nine stages of follicular development which he has defined represent successive stages in a *discontinuous* process of growth, the bulk of the oocyte population of each stage undergoing atresia, and only a minute proportion reaching maturity. At fixed intervals a number of follicles belonging to the most primordial group become active and, presumably developing extremely rapidly, become part of the population of the next phase of growth. Simultaneously a proportion of the existing population of each phase replaces a proportion in the next phase of development. Desaive estimates that for every six follicles passing through the final stage of development in the rabbit ovary, 1440 follicles have to pass through the stage which follows the primordial phase of development. Any one ovary contains follicles representing twenty pulses of follicular development. A complete ovarian cycle he estimates to last 28 days—an interval of time which, like the 11 days estimated for the complete development of a single follicle, bears no obvious relation to any other periodic process in the rabbit, an animal which ovulates as a rule only under the stimulus of mating (Smelser, Walton & Whetham, 1934). Thyroidectomy and hypophysectomy neither interrupt nor alter the periodicity of the process, but only eliminate the final phases of follicular development.

The observations assembled here do not bear on Desaive's mathematical treatment of follicular growth. They do, however, throw doubt on the fundamental assumption that at any given moment the different phases of follicular development are in equilibrium, that is to say, on the assumption that there is a fixed numerical relation between the numbers of follicles in different stages of growth. Desaive claims that the stages of follicular development which he has defined are in no sense arbitrary, since each represents a measure of size to which some follicles correspond at any given moment. Our own classification is in terms of layers of granulosa cells, but the stages we have defined, while they also unquestionably relate to given phases of follicular growth, are in our view completely arbitrary. Each could nevertheless be characterized by some dimensional term (e.g. diameter or volume) whose variance could also be readily established. There is, therefore, no theoretical difference between Desaive's and our own characterization of the stages of follicular growth. The fact that there does not appear to be any constant relation, as shown by a χ^2 analysis, between the numbers of follicles in different stages of growth in the rat and monkey thus suggests that the same precise laws of follicular growth deduced by Desaive for the rabbit do not necessarily apply to other species.

The only alternative to such a conclusion is to follow Desaive further in his analysis, and to assume first, that all variation in the proportion of follicles in different stages of development (i.e. in the 'follicle-spectrum') has some specific biological significance; and second, that it is possible to relate any given follicle-spectrum to one or other of the finite number of 'pulses' of follicular growth which, according to him, together comprise a complete ovarian cycle (twenty in the rabbit). Desaive's treatment presupposes, however, that the variation which occurs in the size of the follicle in each of his stages is unimportant in the statistical sense, and further, that the follicular sizes of successive phases do not intergrade—a conclusion which emerges directly from his basic tables. We have no evidence that the latter is the case for either the rat or the monkey. On the contrary, our own observations, like those of many other workers (e.g. Paesi, 1949), imply a continuous, not a discontinuous process of follicular growth. Follicles cannot be classified in size groups separated by gaps representing sizes which do not occur. Further, our own studies provide every indication that variation, in its statistical sense, cannot be disregarded in the study of ovarian function. It is practically certain that Desaive's analysis would have yielded less precise results if his methods had taken full account of this factor.

SUMMARY

The relative proportion of oocytes in different stages of development has been studied in the rat and monkey. Oocytes surrounded by an incomplete cellular cover or by a single layer of cells generally make up 90 % or more of the total present, while the frequency with which the larger follicles occur decreases inversely and exponentially with size. Contrary to what has been stated to be the case in the rabbit, there does not appear to be any constant relation, as shown by a χ^2 analysis, between the numbers of follicles in different stages of growth in the rat and monkey.

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REFERENCES

Desaive, P. (1940). Contribution radio-biologique à l'étude de l'ovaire. *Arch. Biol., Paris*, **51**, 587–922.

Desaive, P. (1947). Contribution à l'étude du mécanisme de l'évolution et de l'involution folliculaires dans l'ovaire de lapine adulte. *Arch. Biol., Paris*, **58**, 331–446.

Desaive, P. (1948). Contribution à l'étude du mécanisme de l'évolution et de l'involution folliculaires dans l'ovaire de lapine adulte. (Deuxième partie.) *Arch. Biol., Paris*, **59**, 31–146.

Green, S. H. & Zuckerman, S. (1951). The number of oocytes in the mature rhesus monkey (*Macaca mulatta*). *J. Endocrinol.* **7**, 194–202.

Mandl, A. M. & Zuckerman, S. (1950). The numbers of normal and atretic ova in the mature rat. *J. Endocrinol.* **6**, 426–435.

Mandl, A. M. & Zuckerman, S. (1951*a*). Numbers of normal and atretic oocytes in unilaterally spayed rats. *J. Endocrinol.* **7**, 112–119.

Mandl, A. M. & Zuckerman, S. (1951*b*). The relation of age to numbers of oocytes. *J. Endocrinol.* **7**, 190–193.

Paesi, F. J. A. (1949). The relation between the rate of growth of the ovarian follicles and the shape of the frequency curve representing their variability in size. *Acta endocrinol.* **3**, 173–180.

Smelser, G. K., Walton, A. & Whetham, E. O. (1934). The effect of light on ovarian activity in the rabbit. *J. exp. Biol.* **11**, 352–363.

THE FACTORS GOVERNING THE OPENING OF THE EYES IN THE ALBINO RAT

By J. T. EAYRS
Department of Anatomy, University of Birmingham

The eyelid primordia appear in the rat about the 17th day of intra-uterine life. A day later, their epithelial margins adhere, the process of junction spreading from the canthi towards the centre. Disjunction normally begins at about the 13th day of post-natal life, commencing in the centre and spreading outwards towards the canthi.

Our knowledge of the factors underlying the disjunction process consists of a series of inferences derived from a study of the associated histological changes. Many factors have been cited as being responsible for the disjunction, and there is general agreement that a modification of the structure of the junction epithelium is the most important. Contino (1907), who studied the process in the human foetus, was of the opinion that the secretion from newly matured sebaceous and tarsal glands was responsible for the softening and destruction of this epithelium. This view was supported by the later studies of Alferow (1931) on the cat and dog, but not by those of Dordi (1947) (cat and mouse). Addison & How (1921) proposed that proliferation from the stratum germinativum caused the death of centrally situated cells by displacing them from their source of nutrition, and that the subsequent separation of the lids was assisted by the outgrowth of the eyelashes from newly developed follicles. Mechanical tension has also been suggested as a contributory factor, exerted either by the action of the levator palpebrae muscles (Contino, 1907), or by the pressure of secretion of the glands of Zeiss (Ask, 1908). Burrows (1944), pointing to the similarity between the adhesion and the subsequent separation of epithelia in many parts of the developing organism, suggested that the process of keratinization might be controlled by the secretion of some unspecified hormone.

The present experiments were undertaken to determine the possible part played by certain endocrine, nervous and mechanical factors in effecting the disjunction of the eyelids, and the subsequent opening of the eyes in the albino rat.

MATERIALS AND METHODS

(i) *Influence of sex hormones*

The infant rats used in this experiment were bred from females derived from a dealer's stock. Breeding was arranged so that litters were born in pairs on the same day. On the day of birth, half of one of these litters was selected at random and interchanged with half of the other in equal numbers of each sex, any odd rat being discarded. Each member of one of the two reconstituted litters was then treated daily with a sex hormone dissolved in a mixture of 1 part arachis oil to 2 parts ether. A drop of this solution, amounting to a daily application of 100 μg. of testosterone propionate or 3 μg. of oestradiol diacetate, was placed on both eyelids daily and gently rubbed in with a glass rod. The other reconstituted litter was treated with a similar amount of the solvent only. The treatment was discontinued as soon as the eyelids began to separate.

The onset of disjunction is usually marked by the appearance of a central pinhole in the junction epithelium. This enlarges until the palpebral fissure attains its full width. The progress of disjunction was followed quantitatively by allotting scores for its successive stages. One point was given for the appearance of the pinhole, and three for the fully separated eyelid. Any intermediate stage was scored as two points. The eyelids were examined daily after the 10th post-natal day at 9 a.m., 3 p.m. and 11 p.m., and at the end of the experiment the scores allotted to each of the two groups of litter-mates within each reconstituted litter were totalled and expressed as a percentage of the score attained when all eyes were fully open. These percentages were then plotted against time, and the time at which each group of litter-mates gained 50 % of the possible score was read off from the graphs. These readings gave an estimate of the mean time at which the eyes opened in normal rats and in their hormone-treated litter-mates. The differences between these means were tested for statistical significance.

At the end of each experiment all animals were weighed to the nearest 0·5 g., and the mean weights of the experimental and control animals in each litter were calculated and compared. Correlation coefficients were calculated to test whether the differences in the mean time of disjunction were correlated with differences in mean body weight (Fisher, 1946).

(ii) *Influence of thyroid hormone*

The effects of thyroid deficiency were ascertained from observations made during a concurrent experiment. A similar technique of interchanged litters was used. For the first 5 days of life the experimental animals received daily subcutaneous injections of 0·05 ml. of a 4 % suspension of methyl thiouracil in a 2 % solution of sodium alginate, and thereafter 0·10 ml. daily. The controls were injected with a similar amount of the sodium alginate solution only. The eyelids of the experimental and control animals were examined at 14 days old.

(iii) *Influence of sympathetic innervation*

Unilateral cervical sympathectomy was performed under ether anaesthesia in twenty-five rats aged between 5 and 7 days. The submaxillary gland was retracted and a small incision in the cervical fascia made at the anterior border of the omohyoid muscle. This was widened with forceps to expose the carotid sheath. The superior cervical ganglion was freed from the internal carotid artery as far cranially as possible, and was then removed. A mock operation was performed on the opposite side. The disjunction process was scored for both eyes as in the first experiment. Differences in the behaviour of the sympathectomized and control eyelids were measured in terms of the first appearance of disjunction, and of the duration of the disjunction process. At 30 days old, the relative width of the two palpebral fissures was compared and the animals were then killed. The tissues on the operated side of the neck were dissected out and examined in serial sections for remnants of the superior cervical ganglion.

In some animals, the superior cervical ganglia were removed bilaterally in order to determine whether an earlier separation of the eyelids on the intact side of the unilaterally sympathectomized rat could be responsible for accelerating the disjunction process on the other. In this experiment, mock operated litter-mates were used as controls.

(iv) *Influence of maturation of the visual pathway*

Maturation of the visual pathway was prevented in twenty-six rats by crushing the optic nerve. When this is done carefully the eyeball continues to grow normally. The operations were performed under ether anaesthesia when the rats were 5–7 days old. A small oblique incision in the skin was made about 3 mm. inferior to the temporal canthus. Through this incision, the fascia of the eyeball was placed under slight tension, and pierced with a discission knife. The opening in the capsule was then widened, and the optic nerve located with the aid of a beam of light directed through the cranium. A pair of fine curved forceps was then passed round the back of the eyeball and compressed on the nerve. In some rats this operation was performed unilaterally, with a mock operation on the opposite side. In others a bilateral operation was performed, using bilaterally mock operated litter-mates as controls.

The separation of the lids was recorded as in the previous experiments. Later the animals were killed, and the success of the operation was determined by removing the brain and inspecting the condition of the optic nerves.

(v) *Mechanical factors*

If the optic nerve and its sheaths are cut instead of crushed, the eyeball fails to grow and its place becomes partly occupied by an increased amount of fatty tissue and by an apparent expansion of the Harderian gland. The total volume of the orbital contents is, however, smaller than when a growing eyeball is present, and this results in the overlying eyelid becoming 'slack'. Consequently, any possible effect of traction on the lids or of pressure such as would result from a growing eyeball or from an increased secretory activity of the tarsal glands is minimized.

In twenty rats aged 5–7 days the optic nerve was cut unilaterally using the approach already described. In one litter, the eyeball was removed completely as an alternative procedure. The progress of disjunction was recorded as in the previous experiments. At 30 days old each animal was killed, and its head removed, fixed, and decalcified. A comparison of the eyelids on the experimental and control sides was made from representative coronal sections through the orbits.

RESULTS

Influence of hormones

Treatment with androgen, oestrogen or methyl thiouracil led to a significant retardation in the time of eye opening. The midway point in the disjunction process occurred $9{\cdot}5 \pm 2{\cdot}51$ hr. (standard error of mean of observations on five litters) later in animals treated with androgen ($P = 0{\cdot}01$–$0{\cdot}02$) and $12{\cdot}5 \pm 4{\cdot}15$ hr. (5) later in the oestrogen-treated animals ($P = 0{\cdot}0 > -0{\cdot}05$). The two groups of experimental rats, however, did not grow as well as did their controls. There was a high negative correlation between the retardation in body weight and the time of eyelid disjunction in the androgen ($r = -0{\cdot}939$; $P = 0{\cdot}01$–$0{\cdot}02$), but not in the oestrogen-treated rats ($r = -0{\cdot}362$; $P > 0{\cdot}1$).

Another positive effect of the sex-hormone treatment, and one which proved that the hormones got into the systemic circulation, was an abnormal development of the external genitalia. This was particularly conspicuous in the female. The treatment

with androgen led to an increase in the size of the clitoris while that with oestrogen led to so great an enlargement of the vulva as to resemble superficially the scrotal swellings of the male. Both hormones caused deep pitting of the vaginal opening, and in one litter the vagina became patent at 13 days of age.

The injection of methyl thiouracil caused a significant retardation in disjunction. On the 14th day of age, the eyes of fifty-nine out of eighty-nine control rats were open while none of the experimental animals showed any sign of disjunction at that age. There was no significant difference in the mean weights of the control and experimental groups.

Nervous influence

(i) *Unilateral sympathectomy*

Separation of the eyelids on the operated side of the unilaterally sympathectomized rats began $14{\cdot}4 \pm 3{\cdot}00$ hr. (25) later than on the intact side ($P < 0{\cdot}01$). On the other hand, the whole process of disjunction of the sympathectomized eyelids took a significantly shorter time ($9{\cdot}3 \pm 2{\cdot}57$ hr.; $P < 0{\cdot}01$) than did that of the controls. These changes were usually associated with a difference in the mechanism of disjunction. After sympathectomy, the pinhole stage of opening was less frequently seen than in normal animals ($\chi^2 = 4{\cdot}95$ with one degree of freedom; $P = 0{\cdot}02$–$0{\cdot}05$), disjunction usually starting as a fenestration of the whole width of the junctional epithelium. But when a pinhole did appear, the epithelium was frequently found to be completely broken down at the next examination. In spite of the completed disjunction, the sympathectomized eyelids were often kept closed for several days, opening only when the animals were picked up. At 30 days of age, however, they were usually, but not always partly open.

Post-mortem examination of serial sections through the neck tissues showed that in most animals a small piece of the superior cervical ganglion at the base of the skull had escaped removal.

(ii) *Bilateral sympathectomy*

Separation of the eyelids was retarded in bilaterally sympathectomized animals. The first appearance of disjunction occurred $13{\cdot}6 \pm 6{\cdot}46$ hr. (20) later than in the controls, the difference being statistically significant ($t = 2{\cdot}129$; $P = 0{\cdot}02$–$0{\cdot}05$). Post-operational mortality was very high, and those animals which survived grew slowly. Hair growth was impaired, the animals being sparsely covered with fine hair through which the skin could be seen. They were otherwise healthy and active. These differences in growth were not observed either in the mock-operated or in the unilaterally sympathectomized rats.

(iii) *Destruction of the visual pathway*

Attempts to crush the optic nerve were not uniformly successful. At autopsy some rats were found to have two normal-sized eyeballs, only one of which had an intact (and presumably functional) optic nerve the other having disappeared. Analysis of the data relating to these rats failed to show any difference between the intact and operated sides either in the time of first appearance of disjunction or in the duration of the disjunction process. Other rats were found to be completely blind, having no optic nerve on either side. At least one eyeball was of normal size however, the other

being atrophic. In order to eliminate any influence due to mechanical factors only the data relating to the separation of the lids covering normal-sized eyeballs were analysed and compared with similar data for mock-operated litter-mates. The analysis showed that there was no difference between the blind and intact animals in the time when the eyelids began to separate, but the disjunction process in the blind rats took $4{\cdot}4 \pm 3{\cdot}14$ hr. (26) longer than in the controls. This difference, however, was of doubtful statistical significance ($t = 1{\cdot}410$, $P = 0{\cdot}1$–$0{\cdot}2$).

With minor exceptions, the eyelids covering normal-sized eyeballs, whose optic nerves had degenerated, were kept as widely open at 60 days of age as were those of the controls, whether the animal was blind in one or in both eyes.

It may be concluded from these experiments that the autonomic nervous system plays a small but significant part in regulating the opening of the eyes in the rat. On the other hand, stimuli travelling in the visual pathways do not appear to determine the time at which the eyelids separate, nor do they regulate the extent to which the eyes are ultimately kept open.

Influence of mechanical factors

The disjunction process began on the operated side in only one of twenty rats in which one eyeball was absent or so grossly atrophic that its presence was negligible. Histological examination of the experimental and control eyelids at the end of the experiment showed that, although certain morphological changes had taken place in the structure of the lids, the cornification of the interpalpebral epithelium was complete and did not differ in appearance from that of the control tissues.

It may therefore be concluded that disjunction was delayed, though not prevented, by reduction of the orbital contents.

DISCUSSION

Two processes are concerned in the opening of the eyes: the breakdown of the junctional epithelium and the retraction of the lids to expose the eyeball. While normally concurrent, these events do not seem to be dependent on each other, for when the volume of the orbital contents is reduced either by cutting the optic nerve or by removing the eyeball, disjunction is completed, although the lids are never drawn apart. The factors underlying the two processes are therefore considered separately.

Breakdown of the junctional epithelium

The local application of sex hormones seems to delay the breakdown of the junctional epithelium, an effect which is the reverse of that of sex hormones on epithelia in other parts of the body (e.g. the opening of the vagina, and the separation of the prepuce from the glans penis (Hall, 1938), a process which, from the description of Burrows (1944), is very similar to that of eyelid disjunction). The delay could either be regarded as a direct effect of the sex hormones upon the mechanism of disjunction, as an indirect consequence of the retarded body growth of the young (Biel, 1939), or as due to both factors. Retarded body growth would not appear to have played any significant part in the oestrogen-treated animals, since in them it was poorly correlated with the delay in disjunction. In so far as this implies that the delay was a direct effect, it is of interest that the growth of the hair and eyelashes is

closely associated with the disjunction process (Addison & How, 1921), and that oestrogen inhibits hair growth (Emmens, 1942; Hooker & Pfeiffer, 1943).

Such influence as androgen had appears to have been indirect, since in these experiments the delay in disjunction was closely correlated with a fall in body weight. Since, however, the eyes of the intact female open earlier than those of the male (Donaldson, 1924), it is doubtful whether any physiological significance should be attached to the difference which the present experiments reveal in the behaviour of the male and female sex-hormone.

The retraction of the eyelids

The changes which followed sympathectomy are consistent with those which would be expected to result from a reduced amount of traction on the eyelids. The thinnest part of the interpalpebral lamina, and therefore the zone of greatest weakness, is in the centre of the line of junction (Dordi, 1947). Under tension this would break down first, forming a pinhole which further stretching would cause to widen laterally. In the absence of such tension the tendency would be for a more uniform disintegration throughout the lamina. As a result one would expect not only a later appearance of the first outward signs of disjunction, but also a shorter time between the first appearance and completion of the whole process.

If the interpalpebral epithelium is split surgically, the eye is not held open, the palpebral fissure sealing again with a serous exudate and re-opening at about the normal time. This observation suggests that during the first days of life there is no traction on the eyelids. The fact that the sympathectomized eyelids separate later than those with an intact nerve supply indicates that the nervous influence responsible for the retraction of the lids is of autonomic rather than of somatic origin. Because small pieces of ganglion were left behind, the present experiments do not permit one to assess the full influence of the sympathetic innervation of the eyelid. Govaerts (1939) has shown that section of preganglionic fibres has only a transitory effect, and that a ganglion thus denervated later assumes an independent control over the structures which it innervates. A recovery of function by the remnants of the superior cervical ganglion would therefore tend to ameliorate the possibly more severe effects of complete ganglionectomy.

The factors controlling the disjunction process

According to Walls (1942) the time of disjunction of the eyelids 'always coincides closely with that at which the rods and cones have finished their differentiation'. Since the ganglion cell layer of the retina normally matures earlier than the outer layers, it is to be presumed that at this time the visual pathway has attained functional maturity, as it seems to do in the cat (Tilney & Casamajor, 1924) and in the rat (Addison & How, 1921). Both Walls (1942), who states that the retina matures more rapidly in animals whose eyes are opened prematurely by surgery, and Langworthy (1933), who writes that when the unopened eyelids are sutured unilaterally the part of the optic system associated with the sutured side myelinates more slowly, imply that the maturation of the retina and the disjunction process are causally related. It is, however, clear from the present experiments that this is not the case, in so far as neither unilateral nor bilateral destruction of the optic nerve had

any effect on the time of disjunction. Furthermore, since it was never possible to distinguish blind rats with normal-sized eyeballs from those with intact vision merely by inspecting the extent to which the eyes were kept open, it may be inferred that the activity of the retina does not contribute greatly towards maintaining the tone of the eyelid musculature. It therefore appears that reflex stimulation of muscles of the eyelid by the activity of a newly matured retina is not the cause of disjunction. It is also unlikely that the opening of the eyes accelerates the maturation of the visual pathway, since this matures as rapidly in rabbits reared from birth in total darkness as in normal young (Goodman, 1932). The only factor which the present experiments suggest as significantly concerned is increased tension on the eyelids, attributable, in part at least, to the maturation of autonomic control.

SUMMARY

1. Some factors concerned in regulating the disjunction of the eyelids in the albino rat have been studied.

2. The daily application of both androgen and oestrogen to the eyelids retarded the opening of the eyes. This effect may be related to a concomitant reduction in growth. The effect of sex hormones upon the interpalpebral epithelium was quite different from that upon the epithelium of the genital tract.

3. The daily injection of methyl thiouracil retarded the opening of the eyes without reducing body weight.

4. Unilateral cervical sympathectomy retarded the first signs of disjunction but shortened the process of disjunction and modified the behaviour of the epithelium.

5. The first appearance of disjunction was delayed when the volume of the contents of the orbit was reduced by section of the optic nerve. Even in the absence of a normal eyeball, however, disjunction was complete.

6. The destruction of the optic nerve by crushing was without effect on the time at which disjunction began, and did not modify the extent to which the eyelids were held open.

7. It is concluded from these experiments that the process of disjunction can be divided into two independent but mutually assisting components: the breakdown of the junctional epithelium, and the retraction of the eyeballs. Tension placed on the eyelid mediated through its sympathetic innervation and possibly by pressure exerted from within the orbit plays a part, but seemingly not an essential one, in the disjunction process.

8. The factors which co-ordinate the disjunction process with the maturation of the visual apparatus are discussed.

REFERENCES

Addison, W. & How, H. (1921). The development of the eyelids of the albino rat, until the completion of disjunction. *Amer. J. Anat.* **29**, 1–31.

Alferow, A. (1931). Über Epitelienveränderungen bei Augenspaltenbildung unter den Säugetieren. *Anat. Anz.* **71**, 113–120.

Ask, F. (1908). Über die Entwicklung der Lidränder, der Tränenkarunkel und der Nickhaut beim Menschen, nebst Bemerkungen zur Entwicklung der Tränenableitungswege. *Arb. anat. Inst., Wiesbaden*, **36**, 189–280.

Biel, W. C. (1939). Effects of early inanition on development in the white rat. *J. comp. Psychol.* **28**, 1–15.

BURROWS, H. (1944). The union and separation of living tissues as influenced by cellular differentiation. *Yale J. Biol. Med.* **17**, 197–402.

CONTINO, A. (1907). Über Bau und Entwicklung des Lidrandes beim Menschen. *v. Graefes Arch. Opthal.* **66**, 505–577.

DONALDSON, H. H. (1924). *The Rat: Data and Reference Tables.* Philadelphia: The Wistar Institute of Anatomy and Biology.

DORDI, G. (1947). Il processo di disgiunzione delle palpebre nello sviluppo di alcuni mammiferi. *Arch. ital. Anat. Embriol.* **52**, 136–148.

EMMENS, C. W. (1942). The endocrine system and hair growth in the rat. *J. Endocrinol.* **3**, 64–78.

FISHER, R. A. (1946). *Statistical Methods for Research Workers.* London: Oliver and Boyd.

GOODMAN, L. (1932). Effect of total absence of function on the optic system of rabbits. *Amer. J. Physiol.* **100**, 46–63.

GOVAERTS, J. (1939). Nouvelles recherches sur l'activité spontanée des ganglions sympathiques déconnectés du névraxe. *Arch. int. Physiol.* **49**, 426–448.

HALL, K. (1938). The clitoris of the rat after ovariectomy and the injection of sex hormones. *J. Path. Bact.* **47**, 19–26.

HOOKER, C. W. & PFEIFFER, C. A. (1943). Effects of sex hormones upon body growth, skin, hair and sebaceous glands in the rat. *Endocrinology*, **32**, 69–76.

LANGWORTHY, O. R. (1933). Development of behaviour patterns and myelinization of the nervous system in the human foetus and infant. *Contr. Embryol. Carneg. Instn*, **24**, 1–57.

TILNEY, F. & CASAMAJOR, L. (1924). Myelinogeny as applied to the study of behaviour. *Arch. Neurol. Psychiat., Chicago*, **12**, 1–66.

WALLS, F. (1942). *The Vertebrate Eye.* Michigan: The Cranbrook Press.

THE DEVELOPMENT OF THE HUMAN TRICEPS SURAE

OBSERVATIONS ON THE ONTOGENETIC FORMATION OF MUSCLE ARCHITECTURE AND SKELETAL ATTACHMENTS

By K. W. JAGER and J. MOLL

Department of Anatomy and Embryology, State University, Groningen, The Netherlands

INTRODUCTION

Different aspects of the development of striated musculature have been studied extensively. However, one group of problems has been almost completely neglected: the way in which the individual muscles with their typical structure arise from the original rudiments. We know, for example, almost nothing about the formation of muscle architecture and skeletal attachments.

We studied this aspect of muscle development in the human triceps surae because the complicated structure of the component muscles suggested that transformations, by which the final situation is reached, would be clearly marked.

Some examples of remarkable transformations of the original muscle rudiments have been demonstrated. Schauder (1926) showed the pronator quadratus to be present in the embryonic horse, although the muscle disappears later in connexion with the absence of pronation and supination in this species. Holmgren (1940) described a proximo-distal migration of the human pronator quadratus.

The development of the tendons and their skeletal attachments has been studied mostly from the histological point of view. A migration of the radial insertion of the biceps brachii was found by Holmgren. In an experimental investigation of the head muscles of *Amblystoma*, Hall (1950) found that the muscles develop normally, with regard to their topographical relations, after the removal of the anlagen of the skeletal parts to which they become attached in normal development. This is not in accordance with the deductions of Carey (1922), who supposed that the development of muscles depends directly upon tensions between the areas of origin and insertion caused by skeletal growth. Amprino (1950) found in the eye musculature of the chick an intimate connexion between the development of the muscles and of the structures to which they are normally attached.

Generally, it is supposed that the tendons develop under mechanical influences from the muscles. Hunt (1932) got some results which can only be explained by an independent formation of muscles and tendons. Haines (1932) and Schauder (1924) found in some muscles a relative increase of the tendinous part during development.

The boundaries between the muscular and the tendinous parts of the muscles are much less clearly marked in embryonic stages than later on. It has been established that tendon fibres can be formed from myoblasts (Speidel, 1938; Butcher, 1933).

The development of muscle architecture is almost completely unknown. Only Schauder (1924) studied this problem. He described in the so-called tendon muscles (Sehnenmuskeln) of the horse (muscles in which only a small amount of muscle fibre is present) a gradual increase of the penniform arrangement of the fibres.

The development of the triceps surae has been described by Schomburg (1900) and Bardeen (1907). Their most important findings are the following. The triceps rudiment develops from a common flexor mass. It is situated at the lateral side of the leg. The medial parts of the triceps differentiate after the lateral parts. In this way the tibial origin of the soleus is formed after the fibular, and in the same way the medial origin of the gastrocnemius becomes differentiated after the lateral.

MATERIAL AND METHODS

Twenty human embryos, and a number of still-born foetuses and adult bodies in the dissecting room, were studied.

The embryos varied from 9·5 mm. ($5\frac{1}{2}$ weeks) to 180 mm. (21 weeks) C.R. length. They were studied in serial sections cut transversely, and longitudinally, and stained in most cases with haematoxylin and eosin. Larger specimens were dissected either naked eye or under the dissecting microscope, and in order to differentiate muscle fibres and connective tissue the staining technique described by Tobin & Rumery (1948) was employed.

OBSERVATIONS

9·5 mm. No signs of morphological differentiation of muscle or skeletal tissue are present.

11·5 mm. (*Figs.* 1, 5). Here the skeleton has already partly reached the cartilaginous stage. A morphological differentiation of the muscle tissue is also present. The pre-muscle mass of the leg can already be divided into four parts: the anlagen of the extensors, the peroneal group, the deep flexors and the triceps group. The arrangement of the muscle groups of the leg is shown in Fig. 1 and remains constant during the following weeks of development.

It is remarkable that the triceps does not at this stage occupy its final position, but is situated at the lateral side of the leg. In the proximal and lateral regions muscle fibres have already been formed (Fig. 5). The rudiment joins the distal epiphysis of the femur and the capitulum fibulae.

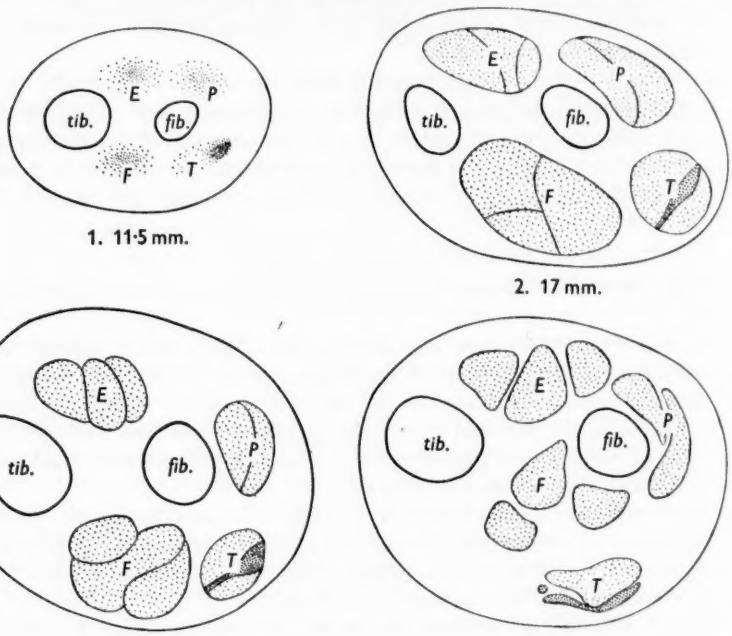
Although a distinction between the parts of the triceps cannot be made, further development demonstrates that the medial part of the blastema (medial to the tibial nerve) gives origin to the medial head of the gastrocnemius. Muscle and tendinous tissue cannot be distinguished at this stage, but the anlage of the tendo Achillis is present. It joins the calcaneum, but has, moreover, a continuation into the sole of the foot.

17 mm. (*Figs.* 2, 6). Both the muscle groups and the individual muscles are now more clearly demarcated (Fig. 2). The triceps still shows its original position at the lateral side of the leg, but a formation of muscle fibres is now taking place in the tibial part also of the gastrocnemius (Fig. 6). The medial part of this muscle has a very diffuse origin from the medial condyle of the femur; the lateral part has a more demarcated origin on the lateral condyle, but is not clearly separated from the origins of the plantaris and the soleus.

The muscle fibres of the gastrocnemius are orientated in a proximo-distal direction. The same applies to the soleus, which originates from the capitulum fibulae. The tendons of insertion of gastrocnemius and soleus, now differentiated histologically, are fused everywhere. They form a tendo Achillis, which is inserted at the plantar

surface of the calcaneum, consists of decussating fibre-bundles and shows a continuation into the sole of the foot as was described for the previous stage.

18 *mm.* (*Figs.* 7, 11). In these specimens the origins of the heads of the gastrocnemius show the same differences as in the former stages; the lateral has a much more clearly defined origin. Between both heads lies a mass of mesenchyme which merges gradually into the heads of the muscle. The size of both heads is nearly the same. The soleus originates from the capitulum fibulae and still has only



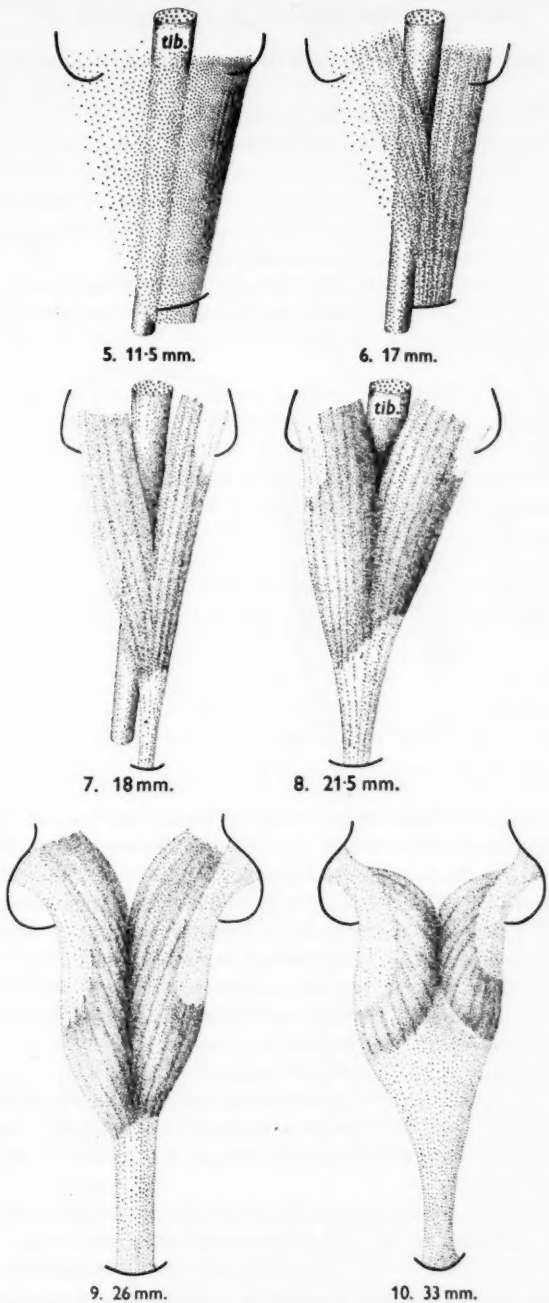
Figs. 1–4. Semi-schematic drawings of transverse sections through the leg of embryos of 11·5, 17, 21·5 and 26 mm. *tib.*, tibia; *fib.*, fibula; *E*, extensor muscles; *P*, peroneal group; *F*, deep flexors; *T*, triceps surae. In Fig. 1 the indistinct borders of the rudiments of the muscle groups are represented by areas of less dense stippling; in Figs. 2–4 the borders of the individual muscles are indicated by lines, and in the same figures tendon rudiments are represented by heavy dots.

a fusiform belly (Fig. 11). The plantaris is clearly discernible for the first time at this stage. It shows a belly which extends farther distally than in the adult.

21·5 *mm.* (*Figs.* 3, 8, 12). By this stage the position of the triceps has already begun to change from the original lateral to the adult dorsal (Fig. 3). The heads of the gastrocnemius have become more independent also. The tibial belly extends farther in a distal direction (Fig. 8), a situation which will become more pronounced during further development.

The soleus shows at the medial margin of its origin a strand of tendon fibres, which was absent in younger stages (compare Figs. 11 and 12).

24 *mm.* No important changes were found. The area of origin of the medial head of the gastrocnemius has reached a more medial position. It is still situated,

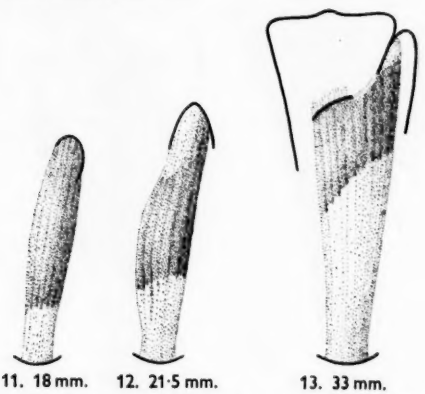


Figs. 5–10. Posterior views of gastrocnemius. Heavy lines, contours of skeletal parts; *tib.* tibial nerve. In Figs. 5 and 6: dotted lines, muscle fibres; heavy stippling, premuscle tissue; light stippling, adjacent mesenchyme. In Figs. 7–10: light stippling, tendons; heavy stippling, muscle fibres.

however, more laterally than is the case in the adult. A broadening of the soleus has taken place.

26 *mm.* Here the orientation of the muscle fibres of the gastrocnemius resembles to some extent that of the adult. In the proximal part of the muscle the longitudinal direction of the fibres has changed into a more oblique one. Both heads of the gastrocnemius show well-defined tendons, but the area of origin of the medial head has not yet reached its final position. This is remarkable, because the lateral head from the beginning possesses its ultimate area of origin.

The area of origin of the soleus extends now from the capitulum fibulae to the adjacent region of the back of this bone. Moreover, the tendon at the medial margin of the muscle has further differentiated.



Figs. 11–13. Posterior view of soleus. Explanations: see Figs. 7–10.

26 *mm.* (***Figs.*** **4, 9**). The individual muscles of the different muscle groups are now completely independent (Fig. 4). The triceps has further extended medially. In comparison with the previous stages a marked lengthening of the tendinous parts of the gastrocnemius has taken place (Fig. 9). The medial head is the larger, as it continues to be. The transformation of the muscle architecture, which was already indicated in the previous stage, has become more pronounced. The fibres now run in an oblique direction from the tendon of origin to the tendon of insertion. It is at this stage that the membranous tendons of origin, situated on the posterior surface of the muscle, are clearly formed for the first time.

30 *mm.* The fibre arrangement of the soleus is still the simple one described in previous stages. A tibial origin has not yet formed, but the strand of tendon fibres at the medial margin of the muscle already resembles the fibrous arch between the two ultimate origins owing to its position behind the posterior tibial vessels and nerves.

The plantaris shows only negligible differences from the adult condition.

33 *mm.* (***Figs.*** **10, 13**). By this stage the extension of the triceps to the medial part of the leg is completed. The gastrocnemius has an external form essentially the same as in the adult (Fig. 10). The muscle architecture, on the contrary, has not yet reached its final pattern. The fibres are only running between those surfaces of

the tendons which face each other, whereas in later stages both surfaces of each tendon serve as areas of insertion and origin.

The soleus for the first time shows a clear but weak tibial origin (Fig. 13). But in addition to the tibial and fibular origins tendon fibres are present at this stage, running from the tibial part of the muscle, posterior to the popliteus, towards the medial condyle of the tibia and the region above the intercondylar fossa of the femur. The muscle architecture has also changed but is still simpler than in the adult. The strand of tendon fibres, which in the younger stages was running along the tibial margin of the muscle, has become the fibrous arch between the tibial and fibular heads. The tendon of insertion, situated on the posterior surface of the muscle, is already continued into the interior of the muscle (compare the final situation shown in Fig. 14).

The tendo Achillis—formerly attached to the plantar surface of the calcaneum—has now gained an insertion more on the posterior surface of this bone. The continuation of the tendon into the sole of the foot has almost disappeared.

35 *mm.* In the soleus the tendinous lamina forming the continuation of the tendon of insertion into the interior of the belly of the muscle runs in a lateral direction at this stage. In view of the fact that the lateral part of the accessory fasciculus* is more strongly developed than the medial, this is remarkable, because the opposite condition is found in the adult, where the tendinous lamina bends intramuscularly towards the medial side, and the medial part of the accessory fasciculus is more strongly developed (Fig. 14).

37, 43, 47 *and* 50 *mm.* In these stages the tibial origin of the soleus becomes more pronounced, but the tendon fibres running from the medial part of the muscle towards the medial condyle of the tibia and to the region above the intercondylar fossa are still present.

55 *and* 69 *mm.* When we compare this stage with the previous ones it is apparent that the medial parts of both gastrocnemius and soleus have increased more than the lateral. Furthermore, we find in this and older stages an attachment of fibres of the gastrocnemius to both sides of the tendons.

The tibial origin of the soleus has become more extended and the accessory fasciculus is more strongly developed. The tendinous lamina, to which the fibres of the accessory fasciculus are attached, has become larger and is visible now at the deep surface of the muscle.

It is interesting (and comprehensible in connexion with the more pronounced growth of the medial part of the muscle) to note that this tendinous lamina is directed now towards the medial side of the leg (compare description of 35 mm. stage).

92 *and* 180 *mm.* (*Fig.* 14). Only minor differences from the previous stages are present. The areas of origin of the soleus on both the tibia and the fibula have extended in a distal direction. The accessory fasciculus and its tendon of insertion have developed still more.

Final condition (new-borns and adults). Here we nearly always find parts of the gastrocnemius originating from the posterior side of the tendons, but the size of these parts is variable and in some cases they are completely absent.

* By accessory fasciculus is meant that part of the soleus which originates from the anterior surface of the tendons of origin (see Fig. 16*d*).

The tendons of origin of the soleus show a marked variability, but in most cases we find two strong tendons, tibial and fibular, which are connected by the fibrous arch. The variations in size of both tendons are related to the position of the accessory fasciculus, which itself is also variable in size.

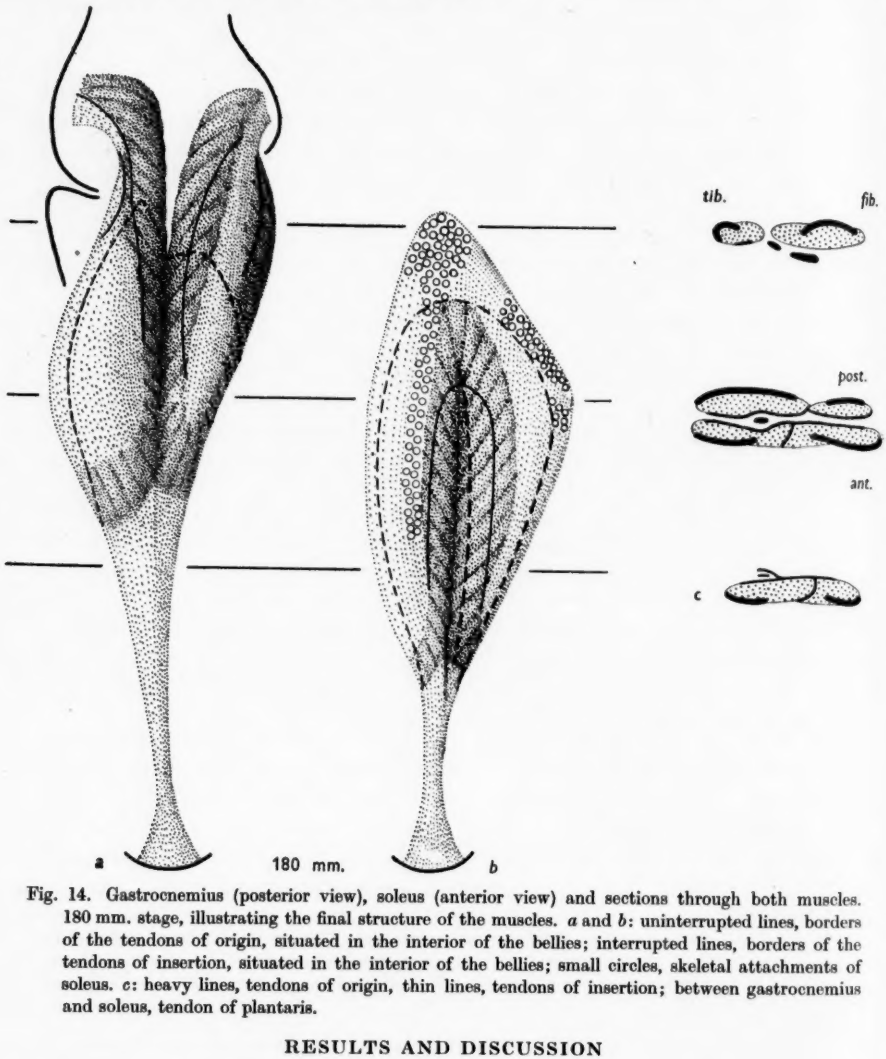


Fig. 14. Gastrocnemius (posterior view), soleus (anterior view) and sections through both muscles. 180 mm. stage, illustrating the final structure of the muscles. *a* and *b*: uninterrupted lines, borders of the tendons of origin, situated in the interior of the bellies; interrupted lines, borders of the tendons of insertion, situated in the interior of the bellies; small circles, skeletal attachments of soleus. *c*: heavy lines, tendons of origin, thin lines, tendons of insertion; between gastrocnemius and soleus, tendon of plantaris.

RESULTS AND DISCUSSION

Development of the external form of the triceps surae

The morphological development of the triceps surae starts with the appearance of a separate rudiment of this muscle group. Contrary to the observations of Schomburg (1900) and Bardeen (1907), we found in the youngest stages no common flexor

mass, but four already independent muscle anlagen (extensors, peroneal group, deep flexors and triceps).

The rudiment of the triceps surae is situated—as was already mentioned by Schomburg and Bardeen—at the lateral side of the leg, not behind the deep flexors as is the case in the adult, but at their lateral side. On the contrary, the last-mentioned muscle group and the others of the leg show their ultimate position from their first appearance.

Originally the muscle rudiment joins the skeleton over a large area. When more clearly demarcated and histologically more differentiated skeletal attachments appear, the rudiment becomes divided into its individual muscles.

A number of factors contribute to the changing of the original position of the triceps into the adult one (first indicated in the 21·5 mm. embryo). In the first place a rapid increase in size of the medial head of the gastrocnemius occurs. In young stages the development of this part of the muscle lags behind. However, a real migration in a medial direction of parts of the muscle situated originally at the lateral side—as suggested by the work of Schomburg and Bardeen—does not take place. From the very first stages a medial part of the anlage is present, although a clear distinction of two heads is not yet possible. The soleus, on the contrary, shows a real extension in a medial direction. This contributes also to the changing of the position of the triceps surae as a whole.

An interpretation of the remarkable shifting of the bulk of the triceps from a lateral to a posterior position—remarkable because such processes are absent in the other muscle groups of the leg—is possible, when one takes the comparative anatomy of this muscle group into consideration. In lower classes of tetrapods the triceps is less strongly developed and is, moreover, situated quite laterally. A medial part of the soleus originating from the tibia is only indicated in anthropoid apes (Kohlbrugge, 1897; Frey, 1913; Fick, 1925). A medial head of the gastrocnemius is more common, but in most cases is weaker than the lateral head.

It is conceivable that in the human embryo the rudiment of the triceps is formed in the same region where the muscle is situated in most tetrapods. The medial extension of the muscle can be interpreted as a means by which the muscle reaches the greater size and the greater area of origin, which is so characteristic for the human triceps surae in connexion with its functional significance in erect posture and locomotion. Our interpretation of the ontogenetic changes in the topography of the triceps finds some support in the observations of Schauder (1926), mentioned in the introduction. The disappearance of the pronator quadratus during the ontogeny of the horse is another example of muscle development in which a muscle achieves the ultimate condition characteristic of the particular species at a later stage.

Muscle architecture and skeletal attachments

Originally all the muscle fibres of the triceps are arranged in a proximo-distal direction. Distinct tendons are absent in these early stages. They are formed from the 17 mm. stage onwards. Like other developmental processes this happens first at the lateral side. The relative size of the tendons increases rapidly, and at the same time the arrangement of the muscle fibres changes. They acquire a more oblique direction, running between those surfaces of the aponeurotic tendons which face each other.

These tendons extend gradually on the surface of the bellies of the muscle. In this way the increasing mass of fibres acquires a much greater area of origin and insertion. Further development does not bring about any considerable changes in the muscle architecture of the gastrocnemius. However, we find a further increase in the area from which muscle fibres originate, when they become attached in later stages to both surfaces of the tendons of origin.

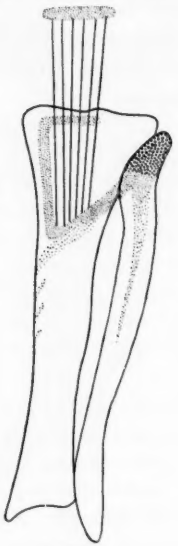


Fig. 15

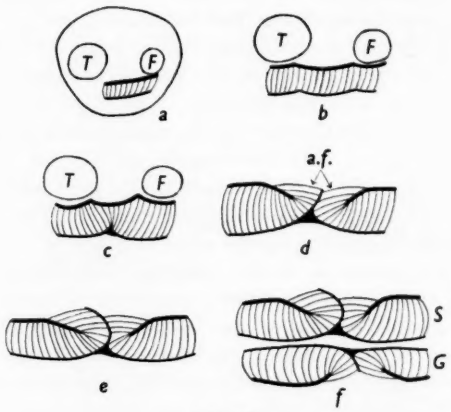


Fig. 16

Fig. 15. Diagram of changes in skeletal attachment of the soleus, posterior view. Heavy dots, original attachment; dense stippling, extension of skeletal attachment; sparse stippling, final extension of skeletal attachment. Further explanation in text.

Fig. 16. Diagram of development of muscle architecture in the soleus. Muscle fibres (projected into the plane of sectioning) are represented by thin lines, tendons by heavy lines. *T*, tibia; *F*, fibula; *S*, soleus; *G*, gastrocnemius; *a.f.* accessory fasciculus of soleus. *a*, transverse section at the level of the fibular origin of the soleus. Tibial origin still absent. *b*, transverse section at the level of the fibrous arch. Both origins present. *c*, transverse section at the same level. Formation of the tendinous lamina of the tendon of insertion already indicated. *d*, transverse section below the level of the fibrous arch. Accessory fasciculus present. Tendinous lamina of the tendon of insertion has increased in size. *e*, transverse section at the same level. Accessory fasciculus and tendinous lamina fully developed. *f*, soleus and gastrocnemius both represented in sections half-way between origin and insertion to illustrate the similarity in ultimate structure.

The soleus shows a somewhat more complicated development. Before changes in the arrangement of the muscle fibres take place, the muscle extends in a medial direction and a strand of tendon fibres, running medially from the capitulum fibulae, is formed.

In the first stages in which a tibial origin is found, the medial part of the muscle shows, moreover, tendinous connexions with medial parts of the head of the tibia and distal parts of the femur (indicated by lines in Fig. 15). These are only present for a short time. They disappear gradually when the tibial origin becomes stronger.

After the appearance of the tibial origin the tendon of origin becomes divided distally into two parts, which are connected by the well-known fibrous arch and attached (compare Fig. 16*d*) to the tibia and to the fibula. In this way two muscle-masses, each with a tendon of its own, are formed. When in later stages muscle fibres are attached to both the deep and the superficial surface of the tendons (we encountered the same process in the gastrocnemius), the accessory fasciculus is formed. It acquires a special tendon by the formation of a continuation of the tendon of insertion into the belly of the muscle (compare Fig. 16*c*, *d* and *e*). In all cases in which this tendinous lamina reaches the deep surface of the muscle, it extends in the adult towards the medial side of the leg (Faller, 1942). In young stages we found the opposite condition. This contrast is quite understandable. In later stages the tibial part of the muscle shows a more rapid increase in size, and this brings about a change in the position of the tendinous lamina, which is naturally directed towards the side from which the greater number of fibres reach it (compare Fig. 16*d* and *e*).

The gradual transformation of the muscle architecture of both soleus and gastrocnemius that we have just described brings about a remarkable similarity in the structure of the two muscles (compare Fig. 16*f*). In both gastrocnemius and soleus the medial and lateral parts show finally the character of a bipennate muscle. Muscle fibres are attached to both deep and superficial surfaces of the tendons of origin (compare Fig. 16*f*).

The skeletal attachments show modifications of different kinds. During early development we find a process of concentration of the skeletal attachments, which is most marked at the origins. In early stages the muscles are attached to the skeleton over an area of considerable size by means of fibres, which closely resemble the fibres of the primitive connective tissue present elsewhere in the leg. These diffuse tendons of origin change gradually into tendons of much smaller size, attached to a less extended area but histologically much more demarcated from the surrounding connective tissue. It seems possible that these transformations depend on the development of the contractile power of the muscles. In the gastrocnemius, for example, the concentration of the tendon of origin occurs first in the lateral head, which is more strongly developed in the first period of development. The development of the tibial origin of the soleus also indicates a development of the tendons directly dependent on the development of the contractile power of the muscle. The presence of tendon fibres running proximally from the medial part of the muscle suggests that the part of the soleus formed by the extension of the muscle in a medial direction causes a longitudinal arrangement of the connective tissue fibres in an area of considerable size. Later on, the skeletal attachment becomes concentrated, and in this way the ultimate origin is formed (compare Fig. 15).

Especially in later stages, on the contrary, some extensions of skeletal attachments occur. An example can be found in both the tibial and fibular areas of origin of the soleus (Fig. 15). In the case of the gastrocnemius the medial head gradually gains a more medial position, but a comparable process is absent in the lateral head.

The development of the common tendon of insertion of the triceps, the tendo Achillis, shows some remarkable features. In young stages the tendon consists of decussating bundles of fibres, an arrangement that has been found in other mammals

also. In some cases the different parts of the triceps show independent but also cross-wise arranged tendons. Different explanations of this arrangement have been advanced, though not very convincingly. Parsons (1894) offered an explanation by means of a totally hypothetical rotation of the foot. Bardeen (1907) and Frey (1913) supposed the cross-wise arrangement to be caused by a migration towards the medial side of the leg of the medial head of the gastrocnemius, originally situated laterally. Having demonstrated that this migration does not occur, we cannot accept this explanation. Perhaps an explanation is possible analogous to the experiments of Rissmann (1934), who found a cross-wise arrangement of tendon fibres when an object had been placed in the course of a regenerating tendon. In the youngest stages the tendo Achillis, being continued into the sole of the foot, is to a great extent independent of the anlage of the calcaneum. It may be that in these stages the calcaneum can be compared with the object which in Rissmann's experiments was placed in the course of regenerating tendons. This hypothesis agrees with the observations of Frey, that the medial part of the soleus, which is formed in later stages of development when the tendo Achillis no longer continues into the sole, has a medial insertion.

The area of the calcaneum, to which the tendo Achillis is attached, changes from a more plantar to a more proximal one in later stages. One gains the impression that the growth of the calcaneum leads to an incorporation of the last part of the tendon into this bone.

SUMMARY

1. The development of the human triceps surae muscle has been studied in embryos, still-born foetuses and adults. Special attention was paid to the development of muscle architecture and skeletal attachments.

2. The triceps surae arises from an independent rudiment and a remarkable shifting of the muscle occurs during development. In the youngest stages it has a lateral position, the final position being reached only gradually.

3. The medial head of the gastrocnemius is formed after the lateral one, but a migratory process does not occur in the formation of the medial head. Originally the heads of the gastrocnemius are attached to the skeleton in an area of considerable extent. In later stages these primitive origins become concentrated into the much smaller final skeletal attachment.

4. The soleus shows a marked extension towards the medial side of the leg. The tibial origin develops much later than the fibular. In early development the soleus originates diffusely from an area of great size.

5. The characteristic muscle architecture of the soleus and gastrocnemius is obtained by considerable rearrangement of the fibres and by their increase in number. These arrangements take place in connexion with a transformation of the tendons, originally rather small, into large aponeuroses.

We are very much indebted to Prof. Dr J. Ariëns Kappers for his valuable assistance in the preparation of the manuscript and the illustrations. We want to thank Mr T. Heikens for his drawings. Our thanks go also to Prof. Dr W. A. Mysberg, who kindly put some embryos at our disposal.

REFERENCES

AMPRINO, R. (1950). Les conditions qui règlent l'organogénèse et la croissance des muscles. *Acta Anat.* **10**, 38–80.

BARDEEN, C. R. (1907). Development and variations of the nerves and the musculature of the inferior extremity and the neighbouring regions of the trunk in man. *Amer. J. Anat.* **6**, 259–390.

BUTCHER, E. O. (1933). The development of striated muscle and tendon from the caudal myotomes in the albino rat, and the significance of myotomic cell arrangement. *Amer. J. Anat.* **53**, 177–190.

CAREY, E. J. (1922). Studies in the dynamics of histogenesis. Intermittent traction and concentration of differential growth as a stimulus to myogenesis. XI. The dynamics of the pectoralis major tendon. *Anat. Rec.* **24**, 86–96.

FALLER, R. A. (1942). Zur Deutung der akzessorischen Köpfe des Schollenmuskels. *Anat. Anz.* **93**, 161–179.

FICK, R. (1925). Beobachtungen an den Muskeln einiger Schimpansen. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **76**, 117–141.

FREY, HEDWIG (1913). Der Musculus triceps surae in der Primatenreihe. *Morph. Jb.* **47**, 1–191.

HAINES, R. W. (1932). The laws of muscle and tendon growth. *J. Anat., Lond.*, **66**, 578–585.

HALL, E. K. (1950). Experimental modifications of muscle development in *Amblystoma punctatum*. *J. exp. Zool.* **113**, 355–378.

HOLMGREN, H. (1940). Beitrag zur Kenntnis des Verhältnisses zwischen der Skelettmuskulatur des Unterarmes und dem M. pronator quadratus während der Entwicklung des Menschen. *Anat. Anz.* **89**, 1–20.

HUNT, E. A. (1932). The differentiation of chick limb buds in chorio-allantoic grafts, with special reference to the muscles. *J. exp. Zool.* **62**, 57–92.

KOHLBRUGGE, H. F. (1897). Muskeln und periphere Nerven der Primaten. *Verh. K. Akad. Wet. Amst.* **5**, 1–240.

PARSONS, F. G. (1894). The morphology of the tendon Achillis. *J. Anat., Lond.*, **8**, 414–418.

RISSMANN, E. (1934). Durch welche Faktoren wird die Anordnung der Fibrillen im jungen Sehnenregnerat bestimmt? *Morph. Jb.* **73**, 1–20.

SCHAUDER, W. (1924). Die fetale Entwickling der 'Sehnenmuskeln' des Pferdes. *Arch. EntwMech. Org.* **102**, 211–262.

SCHAUDER, W. (1926). Über Entwicklung und Rückbildung des M. pronator quadratus des Pferdes. *Anat. Anz.* **61**, 302–310.

SCHOMBURG, H. (1900). Untersuchung der Entwicklung der Muskeln und Knochen des menschlichen Fusses. Diss. Med., Göttingen.

SPEIDEL, C. C. (1938) Studies of living muscles. I. Growth and repair of striated muscles, as revealed by prolonged observations of individual fibers in living frog tadpoles. *Amer. J. Anat.* **62**, 179–236.

TOBIN, CH. E. & RUMERY, R. E. (1948). A method for staining collagenous tissue in gross anatomical sections. *Anat. Rec.* **101**, 281–290.

THE EFFECT OF THYROID DEFICIENCY INDUCED BY METHYL THIOURACIL ON THE MATURATION OF THE CENTRAL NERVOUS SYSTEM

By J. T. EAYRS and S. H. TAYLOR
Department of Anatomy, University of Birmingham

Thyroid hormone has a beneficial effect on body growth in the congenital cretin, but unless treatment is begun very early, it has little influence on mental development (Lewis, 1937; Brown, Bronstein & Kraines, 1939). In hypothyroidism of later life (myxoedema), it improves both the mental and bodily states (Means, 1948). The clinical evidence thus suggests that thyroid deficiency is associated with changes in the maturation of the central nervous system which cannot later be reversed.

This important inference has not been adequately studied by experimental methods. Benda (1947) examined the tissues of the central nervous system in 'a few experimental animals' about which he gives no details, and observed that the most constantly occurring effects of thyroid deficiency are vascular stasis, oedema, an impairment of myelination and loss of cortical cells. These effects are said to resemble those associated with chronic anoxia, and have consequently been attributed to a lowered tissue metabolism. Similar changes have been reported in the brains of young rats born of thiouracil-treated mothers (Barrnett, 1948).

The present experiment on rats was undertaken to study the problem in greater detail. Observations were restricted to the sensory and motor areas of the cerebral cortex (Krieg, 1946), but were correlated with associated changes in the growth and metabolic rate of the experimental animals and in the time of appearance of stereotyped behaviour.

MATERIALS AND METHODS

Experimental procedure

Twenty-eight female albino rats were mated, and on the day of birth half of the young of each sex was interchanged with half of the same sex from another litter born on the same day, any odd rat being discarded. In this way, two reconstituted litters of equal size and similar composition were formed. One of these reconstituted litters received subcutaneous injections of a 4 % suspension of methyl thiouracil in a 2 % solution of sodium alginate. 0·05 ml. of this suspension was injected daily for the first 10 days of life, 0·1 ml. for the next 10 days and 0·2 ml. for the remainder of the experiment. The other litter, which acted as control, was given a similar amount of the sodium alginate solution only. In order to test whether the experimental treatment was effective, the oxygen consumption of the experimental and control litters was compared at intervals by the method used by Krohn & White (1950).

Tests of behaviour

The air-righting reaction and the placing reflex were used as tests of the maturation of automatic behaviour. Each animal was held back downwards, and was then dropped on to a cotton-wool pad from a height of 1 ft. The air-righting response was recorded as present in those rats which landed on all fours.

The presence of the placing reflex was tested by touching the chin against a metal bar. The mature animal responds by immediately placing its fore-paws on the bar on either side of its chin. If this response did not occur within 3 sec. of applying the stimulus it was judged to be absent.

Preparation of tissues

The rats were weighed at 15 and 24 days of age and were then killed by chloroform. Their tissues were fixed in formalin by the perfusion method (Eayrs, 1950) using the perfusing and fixing fluids of Koenig, Groat & Windle (1945). The brains were

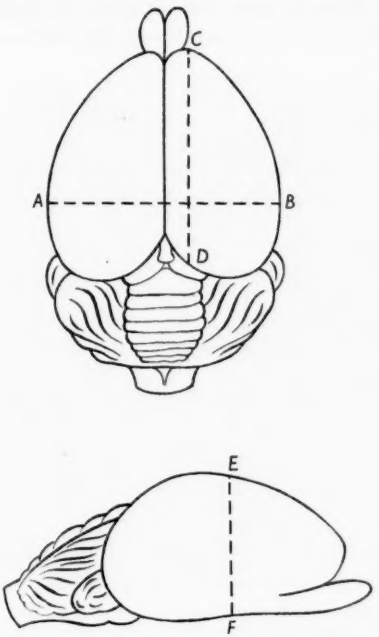


Fig. 1. Linear dimensions used to estimate growth of rat brain (see Table 2). The plane *EF* passes vertically through the optic chiasma.

removed, weighed, and placed in fixative for a week. They were then measured with callipers along the three axes shown in Fig. 1, cut in a standard vertical plane passing through the optic chiasma and the sensorimotor cortical areas and subsequently embedded in paraffin wax. Coronal sections at 7 μ were taken from the cut surface and were progressively stained for Nissl substance in gallocyanin chromalum for 24 hr. at 37° C. (Einarson, 1932).

The trachea with the thyroid gland attached was also removed, embedded in paraffin, cut at 7μ and stained with Ehrlich's haematoxylin and eosin.

Quantitative estimations

Two fine lines were drawn in Indian ink on the coronal sections of the cerebral cortex, one passing horizontally through the most central of a constantly occurring

group of blood vessels lying at the base of the longitudinal fissure and the other vertically through the apex of the centrum ovale (Fig. 2). These lines mark off the approximate extent of the sensorimotor cortex, the lamina ganglionaris forming a broad arc between them. In four adjoining microsopic fields taken from the middle of this arc, the outline of the cell body of each neurone in which a nucleolus was visible was drawn under the projection microscope. The exact magnification of these drawings (approx. 2000 ×) varied slightly between sections, but was accurately determined for each with a stage micrometer. The cross-sectional areas of the cell bodies were measured on the drawings with a planimeter, and after correction for magnification the mean area of each cell body was recorded in square microns. In addition, the method described by Chalkley (1943) was used to estimate the pro-

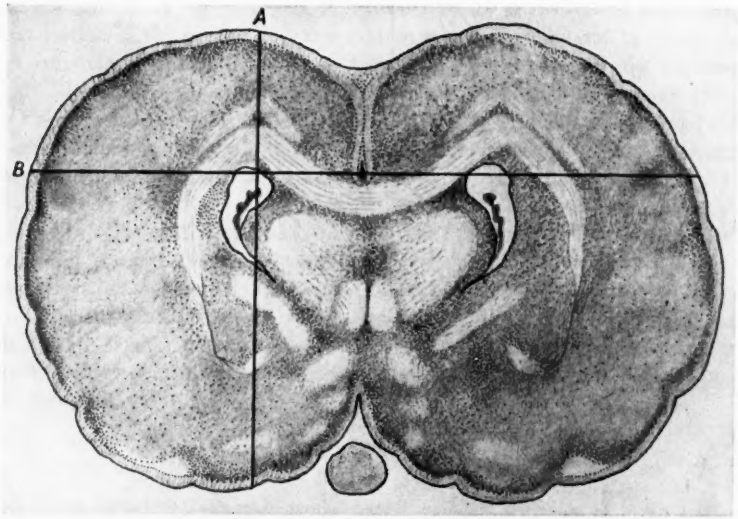


Fig. 2. Coronal section through rat brain passing through optic chiasma. The region of cortex measured extended between the guide lines from *A* to *B*.

portion of the lamina ganglionaris occupied by the pyramidal cell bodies in relation to that occupied by other tissue components. The whole of that part of layer 5 which was contained between the guide lines was measured in this way at a magnification of about 650 ×. Layer 5 has no sharp boundaries, and it is therefore possible that small parts of the adjacent layers 4 and 6 may have been included in some of the measurements. Since, however, this was as likely to occur in the experimental as in the control tissues, there is no reason to believe that any systematic error was involved which would invalidate the results.

These estimations were made on the tissues of six pairs of male litter-mates at both 15 and 24 days of age, and the data were statistically analysed for differences between the experimental and control animals.

RESULTS

Depression of thyroid function

The administration of methyl thiouracil resulted in an early depression in the amount of oxygen consumed by the experimental litters. Fig. 3 shows that at 4 days of age the mean oxygen consumption of the experimental litters had fallen by 18 % compared with that of control litters of the same size. This depression had

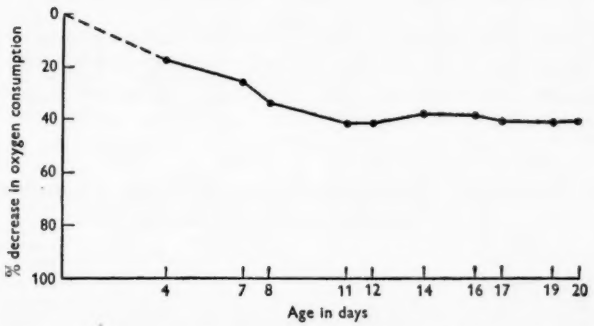


Fig. 3. Depression of oxygen consumption of litters treated with methyl thiouracil from the day of birth. Percentage decrease when compared with litter-mate controls plotted against age.

increased to about 40 % by the 11th day and remained at this level for the rest of the experiment.

The thyroid glands of the experimental animals showed the histological changes which normally follow treatment with thyroid depressant substances (MacKenzie & MacKenzie, 1943).

Growth

The experimental treatment retarded the growth of both body and brain (Table 1). At 24 days old the mean weight of the animals given thiouracil was 11·0 g. less, and that of their brains 0·25 g. less than those of normal litter-mates. Both differences were statistically significant. There was no significant difference in body weight between the two groups at 15 days old, but the bodily proportions of the experimental rats were characteristic of cretinism. The agreement in body weight seemed to be partly due to a disproportionate enlargement of the gut in the thyroid-deficient animals (previously reported by Scow & Simpson (1945) as a consequence of surgical thyro-parathyroidectomy). The brains of the 15-day-old experimental rats weighed significantly less (0·18 g.) than those of the controls.

Comparison of the ratio of brain weight to body weight (Table 1) showed that at 15 days old the bodies of the experimental animals were larger in relation to the size of their brains than were those of the controls. This ratio changed very little in experimental rats between 15 and 24 days of age, but decreased significantly in the controls. It may therefore be inferred that between 15 and 24 days of age the growth of the body was more severely disturbed by the experimental treatment than was that of the brain.

All the linear dimensions measured on the brains of the experimental animals were about 8 % smaller than those of the controls both at 15 and 24 days old. (Table 2). Furthermore, the brains of the thyroid-deficient rats differed from those

Table 1. *Comparison of body weight and brain weight of rats treated from birth with methyl thiouracil and their litter-mate controls*

Measurement	Age (days)	No. of observations	Control	Experimental	Difference	t	P
Mean weight of body (g.) ± standard error of mean	15	32	18·1 ± 0·98	17·1 ± 1·21	1·0 ± 1·67	0·599	0·6–0·5
	24	30	31·8 ± 0·23	20·8 ± 1·43	11·0 ± 2·65	4·155	<0·001
Mean weight of brain (g.) ± standard error of mean	15	32	0·92 ± 0·025	0·74 ± 0·028	0·18 ± 0·037	4·884	<0·001
	24	30	1·08 ± 0·024	0·83 ± 0·020	0·25 ± 0·031	8·004	<0·001
Mean ratio: brain weight × 100/body weight ± standard error of mean	15	32	5·21 ± 0·17	4·46 ± 0·20	0·75 ± 0·265	2·832	0·01–0·00
	24	30	3·60 ± 0·21	4·22 ± 0·25	0·62 ± 0·336	1·880	0·1–0·0

Table 2. *Comparison of linear dimensions of the brains of hypothyroid rats and normal controls at 15 and 24 days old*

Measurement (cm.) ± standard error of mean (see Fig. 1)	Age (days)	No. of observations (litter means)	Control	Experimental	Difference	Percentage difference from control measurement	t	P
Mean width (*AB*)	15	16	1·28 ± 0·020	1·19 ± 0·019	0·09 ± 0·028	7·0	3·290	0·01–0·00
Mean length (*CD*)		16	1·14 ± 0·027	1·03 ± 0·023	0·11 ± 0·035	8·8	2·963	0·02–0·0
Mean height (*EF*)		16	0·83 ± 0·013	0·78 ± 0·010	0·05 ± 0·016	6·0	2·813	0·02–0·0
Ratio: *AB*/*CD*		16	1·130 ± 0·0121	1·155 ± 0·0073	0·025 ± 0·1042	2·2	1·765	0·1 –0·0
Mean width (*AB*)	24	15	1·34 ± 0·014	1·23 ± 0·017	0·11 ± 0·021	8·2	4·993	<0·001
Mean length (*CD*)		15	1·19 ± 0·016	1·06 ± 0·015	0·13 ± 0·022	10·9	6·105	<0·001
Mean height (*EF*)		15	0·85 ± 0·007	0·80 ± 0·007	0·05 ± 0·010	5·9	5·204	<0·001
Ratio: *AB*/*CD*		15	1·123 ± 0·0067	1·161 ± 0·0060	0·038 ± 0·0090	3·4	4·238	<0·001

of the controls in being wider in relation to their length (ratio *AB*/*CD* in Table 2). There also was a difference in the trend of growth, for between 15 and 24 days of age the brains of the control animals grew longer in relation to their width while those of the experimental animals grew wider in relation to their length.

Tests of behaviour

The development of both the air-righting response and the placing reflex was significantly retarded in the experimental animals. The ability to land on their feet was present in more than 10 % of control rats at 15 days old, and in 90 % at 19 days. The corresponding percentages in the experimental animals were 0 and 20 % ($\chi^2 = 43{\cdot}8$ with one degree of freedom; $P < 0{\cdot}001$). Similarly, the placing reflex was elicited in 70 % of the control but in none of the experimental animals at 19 days old.

Development of the cerebral cortex

The mean cross-sectional area of the pyramidal cell bodies in layer 5 of the sensorimotor cortex of thiouracil-treated rats was only two-thirds of that of the controls (Table 3). This difference was present at both 15 and 24 days old, and was statistically significant at both ages. The pyramidal cells of the control animals did not increase in cross-sectional area between 15 and 24 days of age. A slight increase

in the mean size of those of the experimental rats was not statistically significant ($P = 0{\cdot}7$–$0{\cdot}6$).

There were more pyramidal cell bodies per unit area in the experimental tissues than in the control. Statistical analysis (Table 3) shows that this difference was significant at the 5 % level in 15-day-old rats, and at the 1 % level at 24 days of age. Between these two ages, the number of cells per unit area decreased significantly in the control animals ($t = 2{\cdot}582$; $P = 0{\cdot}02$–$0{\cdot}01$), but a relatively small decrease in the thiouracil-treated rats was not statistically significant ($P = 0{\cdot}6$–$0{\cdot}5$).

Table 3. *Comparison of (i) the mean cross-sectional area and (ii) the mean number in a microscopic field of standard size of pyramidal cell bodies in the lamina ganglionaris of the cortex of rats treated with methyl thiouracil and of litter-mate controls*

Measurement	Age (days)	No. of standard fields	Control	Experimental	Difference	t	P
Mean cross-sectional area (μ^2) ± standard error	15	12	260·4 ± 11·20	166·4 ± 11·64	94·0 ± 16·15	5·819	<0·001
	24	12	259·3 ± 4·11	176·3 ± 11·97	83·0 ± 12·58	6·559	<0·001
Mean no. of cell bodies in an area of standard size ± standard error	15	12	24·5 ± 1·72	34·2 ± 3·22	9·7 ± 3·66	2·648	0·05–0·02
	24	12	18·3 ± 1·31	32·0 ± 2·05	13·7 ± 2·44	5·621	<0·001

Table 4. *Comparison of the space occupied by the bodies of the pyramidal neurones of the lamina ganglionaris in relation to that of other constituents; and of the rate of change of this ratio with age*

Measurement	Age (days)	No. of observations	Control	Experimental	Difference	t	P
Ratio: $\frac{\text{intercellular substance}}{\text{pyramidal cells}}$ ± standard error	15	12	4·25 ± 0·115	3·17 ± 0·088	1·08 ± 0·145	7·439	<0·001
	24	12	6·57 ± 0·212	3·71 ± 0·116	2·86 ± 0·242	11·82	<0·001
Regression coefficient: change of ratio $\frac{\text{intercellular substance}}{\text{pyramidal cells}}$ with age	15–24	24	0·257 ± 0·0268	0·060 ± 0·0162	0·197 ± 0·0313	6·311	<0·001

These results show that during the normal growth of the lamina ganglionaris there is a reduction in the number of neurones in a unit area of tissue, while at the same time the size of the cell bodies remains unchanged. The wider spacing of the cell bodies can be attributed to an increase in one or more of the components of the intervening tissue ('intercellular substance'). The inference that this change was much less marked in the tissues of the experimental animals was tested by estimating, in all groups of rats, the volume occupied by pyramidal cell bodies relative to that taken up by the intercellular substance (Chalkley, 1943). Comparison of these measurements (Table 4) shows that, at both 15 and 24 days old, the amount of the intercellular substance relative to the volume occupied by the cell bodies of pyramidal neurones was much (and significantly) less in the experimental animals than in the controls. A plot of this measure against age showed that a significant increase in the relative amount of intercellular substance occurred in both experimental and control animals between 15 and 24 days of age. Regression coefficients were calculated and compared. The rate of increase proved to be significantly greater in the control tissues than in the experimental (Table 4).

DISCUSSION

Changes in growth

While the extent to which body growth was depressed in the present experiments was less than that reported after thyroid deficiency by other workers (Hughes, 1944; Salmon, 1938; Scow & Simpson, 1945), the evidence is clear that the animals given thiouracil were suffering from chronic thyroid deficiency. It is also plain that the treatment depressed the growth of the brain. The two changes do not appear to be interdependent, for while the growth of the body is restored in the thyro-parathyroidectomized rat by treatment with growth hormone, that of the brain is not (Scow & Marx, 1945). It seems therefore that a deficiency of thyroid hormone specifically impairs brain growth.

The brains of the experimental animals were also different in shape from those of the controls. During the first days of life the brain of the normal rat grows more rapidly in length than in width (Sugita, 1918*a*), but this tendency, while present in the controls, was significantly less marked in the experimental animals whose brains were consequently more infantile in general appearance. It is not possible to say whether this change was a direct effect or a secondary result of an impairment to the growth of the skull. Similar, but more severe, changes in the cranio-neural relationship which follow hypophysectomy performed at 6 days old have been attributed to disparity in the rates of growth of brain and skull (Walker, Simpson, Asling & Evans, 1950). The present results suggest that these effects may be largely due to the withdrawal of the thyrotrophic hormone of the pituitary.

Development of automatic behaviour

The appearance of the air-righting response and of the placing reflex were delayed in the thyroid-deficient rats. It is possible that in these animals the stimulus was inadequate to elicit the response, for the reduced excitability of peripheral nerve in hypothyroidism (Horsten & Boeles, 1949) would be expected to be associated with a rise in the threshold of sensory receptors. The fact that the air-righting reflex eventually appeared in the experimental rats without a corresponding increase in the stimulus suggests, on the other hand, that the retarded development of this reaction was due to an impairment to central nervous maturation. It is therefore significant that morphological changes were found in that part of the cerebral cortex whose integrity is essential for the performance of the placing reflex (Brooks, 1933).

Changes in the cerebral cortex

The two major changes which were observed in the maturation of the lamina ganglionaris were in the size and spacing of the cell bodies. Sugita (1918*b*) found that the pyramidal cells grow very slowly in the rat after about the 12th day of age, and this is borne out by the measurements made in the present experiment. The pyramidal cell bodies in the experimental tissues, however, were significantly smaller than in the control. Qualitative observations suggested that this arrested growth involved a reduction in the size of both nucleus and cytoplasm. Since the

outline of the nuclear membrane was frequently obscured by the Nissl substance it proved impracticable to test the inference quantitatively.

The cell body itself, however, is but a small part of the whole neurone, a considerable proportion of which consists of processes, contained in the intercellular substance, which are not visible in Nissl-stained preparations. The reduction in amount and the slower rate of increase of the intercellular substance in the brains of the thyroid-deficient rats probably have a greater functional significance than the smaller size of the cell bodies; for while it is likely that these differences were due in part to a shortage of myelin (Benda, 1947), another possibility is that some change occurred in the development of the axon-dendritic network which is so prominent a feature of cortical maturation (Conel, 1941, 1947). This would imply that a disturbance to the formation of the proper number and pattern of inter-neuronal connexions may occur in hypothyroidism which must undoubtedly be reflected in behaviour and might well account for the retarded mental development of the human cretin. The application of more specific histological techniques to demonstrate the structure of the intercellular substance should enable a quantitative assessment to be made of the nature of the disturbance to this aspect of cerebral maturation.

SUMMARY

1. The changes in the growth of the body and of the brain which follow the administration of methyl thiouracil from the day of birth have been studied in the albino rat.

2. Thiouracil-injected rats grew less well than their litter-mate controls, and were cretinous in their appearance.

3. The brains of thiouracil-injected rats weighed significantly less than those of the controls at both 15 and 24 days old.

4. The experimental animals were retarded in the time of acquisition of the air-righting response and of the placing reflex.

5. The pyramidal cells of layer 5 of the sensorimotor cortex of the experimental animals were smaller than those of the controls. There were more of these cells per unit area of cortex in the experimental rats than in the controls. This was due not only to the smaller size of the cell bodies but also to a reduced amount of intervening substance.

6. The size of the pyramidal cell bodies did not increase appreciably between 15 and 24 days of age, but the amount of the intervening substance did. The rate of increase of this substance was less in the thiouracil-injected animals than in the controls.

7. The significance of these findings in relation to the mode of influence of thyroid deficiency on the development of the central nervous system is discussed.

REFERENCES

Barrnett, R. J. (1948). Quoted in Pincus, G. and Thimann, K. V., *The Hormones*. New York: Academic Press.

Benda, C. E. (1947). *Mongolism and Cretinism*. London: W. Heinemann.

Brooks, C. M. (1933). Studies on the cerebral cortex. II. Localised representation of hopping and placing reactions in the rat. *Amer. J. Physiol.* **105**, 162–171.

Brown, A. W., Bronstein, I. P. & Kraines, R. (1939). Hypothyroidism and cretinism in childhood. VI. Influence of thyroid therapy on mental growth. *Amer. J. Dis. Child.* **57**, 517–523.

Chalkley, H. W. (1943). Method for the quantitative morphologic analysis of tissues. *J. Nat. Cancer Inst.* **4**, 47–53.

Conel, J. L. (1941). *The Post-natal Development of the Human Cerebral Cortex.* Vol. 2. *The Cortex of the One-month Infant.* Cambridge, Mass.: Harvard University Press.

Conel, J. L. (1947). *The Post-natal Development of the Human Cerebral Cortex.* Vol. 3. *The Cortex of the Three-month Infant.* Cambridge, Mass.: Harvard University Press.

Eayrs, J. T. (1950). An apparatus for fixation and supravital staining of tissues by the perfusion method. *Stain Tech.* **25**, 137–142.

Einarson, L. (1932). Method for progressive selective staining of Nissl and nuclear substance in nerve cells. *Amer. J. Path.* **8**, 295–308.

Horsten, G. P. M. & Boeles, J. Th. F. (1949). The influence of hypothyroidism on the excitability of peripheral nerves. *Arch. int. Pharmacodyn.* **78**, 93–99.

Hughes, A. M. (1944). Cretinism in rats induced by thiouracil. *Endocrinology*, **34**, 69–76.

Koenig, H., Groat, R. A. & Windle, W. F. (1945). A physiological approach to perfusion-fixation of tissues with formalin. *Stain Tech.* **20**, 13–22.

Krieg, W. J. S. (1946). Connections of the cerebral cortex. I. The albino rat. A. Topography of the cortical areas. *J. comp. Neurol.* **84**, 221–275.

Krohn, P. L. & White, H. C. (1950). The effect of hypothyroidism on reproduction in the female albino rat. *J. Endocrinol.* **6**, 375–385.

Lewis A. (1937). A study of cretinism in London. *Lancet*, **1**, 1505–1509; **2**, 5–9.

MacKenzie, C. G. & MacKenzie, J. B. (1943). Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. *Endocrinology*, **32**, 185–209.

Means, J. H. (1948). *The Thyroid and its Diseases*, 2nd ed. Philadelphia: Lippincott.

Salmon, I. N. (1938). The effect on the growth rate of thyro-parathyroidectomy in new born rats and of the subsequent administration of thyroid, parathyroid and anterior hypophysis. *Endocrinology*, **23**, 446–457.

Scow, R. W. & Marx, W. (1945). Response to pituitary growth hormone of rats thyroidectomised on the day of birth. *Anat. Rec.* **91**, 227–236.

Scow, R. W. & Simpson, M. E. (1945). Thyroidectomy in the newborn rat. *Anat. Rec.* **91**, 209–226.

Sugita, N. (1918*a*). Comparative studies on the growth of the cerebral cortex. III. On the size and shape of the cerebrum in the Norway rat and a comparison of these with the corresponding characters in the albino rat. *J. comp. Neurol.* **29**, 1–9.

Sugita, N. (1918*b*). Comparative studies on the growth of the cerebral cortex. VI. Part I. On the increase in size and on the developmental changes of some nerve cells in the cerebral cortex of the albino rat during the growth of the brain. *J. comp. Neurol.* **29**, 119–151.

Walker, D. G., Simpson, M. E., Asling, C. W. & Evans, H. W. (1950). Growth and differentiation in the rat following hypophysectomy at 6 days of age. *Anat. Rec.* **106**, 539–554.

NERVOUS PATHWAYS IN THE DEVELOPING HUMAN LABYRINTH

By C. C. D. SHUTE

*Anatomy Department, Middlesex Hospital Medical School, London W.*1

INTRODUCTION

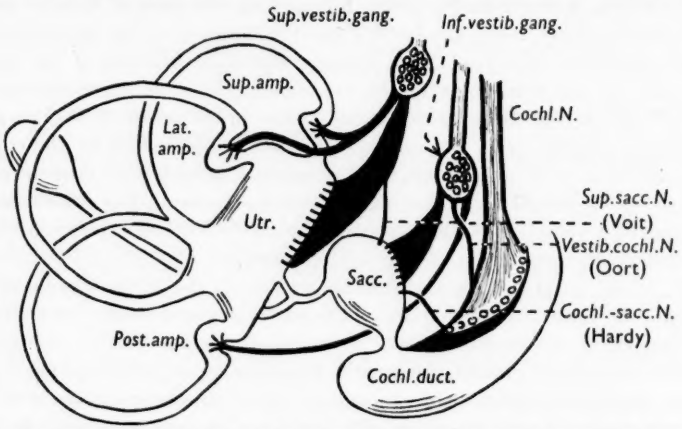
The vast literature devoted to the inner ear does not include an account of the development of the auditory nerve based on human material specifically stained for nerve fibres. Lorente de Nó (1926) followed up the work of Retzius and Cajal with a detailed study, using the latter's silver technique, of the innervation of the labyrinth in mouse embryos. More recently the differentiation of the acoustico-facial ganglion has been investigated experimentally in other vertebrate types. It is now believed that the acoustico-facial portion of the neural crest gives rise only to geniculate ganglion cells. The acoustic ganglion is budded off by the epithelium of the medial wall of the otic vesicle, whose formation according to Trampusch (1942) is induced by the neural crest from the overlying ectoderm. Our knowledge of the later stages of development is derived mainly from the work of Streeter (1907, 1912), who described the separation of the cochlear ganglion from the inferior vestibular ganglion, which, he stated, may still be incomplete in the adult. The facial part of the ganglion he reported as being distinct from the acoustic mass even at the 7 mm. stage. According to Pearson (1947), however, the separation of the geniculate ganglion is a gradual process, frequently resulting in an inner portion being left behind in the internal auditory meatus close to the vestibular ganglion. van Buskirk (1945) also noted sensory ganglion cells beginning to appear as soon as the pars intermedia had joined the motor root of the facial nerve.

Since Streeter's original paper, three components of the auditory complex have been discovered whose derivation and significance have been much debated. They are the superior saccular nerve of Voit (1907), the vestibulo-cochlear anastomosis of Oort (1918) and the cochleo-saccular nerve of Hardy (1934). These small nerves apparently link each of the three parts of the acoustic ganglion with an area of neuro-epithelium which is otherwise foreign to it.

Although it has been generally accepted that Voit's nerve to the cranial end of the saccular macula comes off the superior vestibular ganglion, Poljak (1927) maintained that in the cat its fibres could be traced through an anastomotic bundle to the inferior vestibular ganglion. Also Lorente de Nó (1933) observed that in the mouse all the cells representing the macula of the saccule occupy the same, ventro-caudal part of the vestibular ganglion. Hardy found in 40 % of adult human labyrinths evidence of nerve fibres running to the caudal end of the saccular macula from the region of the basal coil of the cochlear ganglion, and she regarded them as arising most probably from cochlear cells. Weston (1937) suggested that Hardy's nerve might be derived from outlying vestibular ganglion cells which he observed close to the cochlear ganglion in the dog. G. L. Rasmussen (1946), in micro-dissections of cat material, recognized that Hardy's nerve could be traced proximally to the inferior vestibular ganglion. The anastomosis described by Oort between the

inferior vestibular ganglion and the cochlear nerve has been variously interpreted as being vestibular or cochlear in origin. Lorente de Nó (1926) held that the bundle consisted of aberrant cochlear fibres, whereas Poljak (1927) believed it to be vestibular or even sympathetic, and Kappers, Huber & Crosby (1936) considered it important phylogenetically as the mammalian representative of the vestibular nerve to the lagena.

The status of Oort's nerve has been further complicated by the work of Weston (1937) who found in the dog and other mammals two separate bundles, both apparently of vestibular origin. He distinguished (*a*) a nerve, unaccompanied by ganglion cells, running from the junction of the superior and inferior vestibular



Text-fig. 1. Diagram of the right labyrinth, lateral aspect. This is the commonly accepted version of the distribution of the auditory nerve, including the superior saccular nerve of Voit (1907), the vestibulo-cochlear anastomosis of Oort (1918), and the cochleo-saccular nerve of Hardy (1934). The vestibular and cochlear divisions of the auditory trunk, formed by central processes of bipolar acoustic cells, are shaded, while the peripheral branches of the vestibular and cochlear ganglia are shown black.

ganglia to the basal turn of the cochlear duct; and (*b*) a nerve, accompanied by outlying vestibular ganglion cells, running from the inferior vestibular ganglion to the cochlear duct and by a recurrent course to the saccule. Yet another interpretation of the vestibulo-cochlear bundle has emerged from the experimental studies of G. L. Rasmussen (1946) on the course of the so-called olivary peduncle. By means of a modified Marchi technique he showed that in cats a lesion between the facial colliculi in the hind-brain led to a myelin degeneration of a tract of fibres which left the medulla between the divisions of the vestibular nerve to reach the cochlea by way of the inferior vestibular ganglion and the anastomosis of Oort. He therefore concluded that Oort's nerve was not sensory, but consisted rather of visceral efferent fibres, presumably preganglionic, furnishing a secretomotor or vasomotor supply to the cochlea. He was unable, however, to demonstrate any post-ganglionic cells on the course of the nerve or amongst the bipolar cells of the cochlear ganglion.

The commonly accepted version of the auditory complex, based on the work of Voit, Oort and Hardy, is illustrated diagramatically in Text-fig. 1. If Hardy's view

is correct, the saccule receives a supplementary innervation from cochlear ganglion cells. This has been held to support some experimental evidence that the saccule functions with the cochlea rather than as an organ of equilibration. Hardy herself did not exclude the possibility of a vestibular origin for the cochleo-saccular nerve, although she considered it to be distinct from the anastomosis of Oort. Since Oort's bundle is considerably stouter than the nerve of Hardy, the two cannot be in complete continuity, and some at least of the fibres of Oort's bundle must be distributed to the cochlea. Therefore, if the vestibulo-cochlear anastomosis of Oort is a true branch of the inferior vestibular ganglion, the basal turn of the cochlear duct must, like the saccule, be connected with both inferior vestibular and cochlear ganglion cells. This interpretation would bear out further the suggestion that the saccule acts as a vibration receptor.

There have been but few references to variations in the innervation pattern of the labyrinth due to fibre bundles running an aberrant course. Lorente de Nó (1926), in his studies on mice, noted the posterior ampullary nerve at an early stage of development giving off eight or more aberrant branches which either reached sensory epithelium by a devious route or lost themselves in connective tissue. All were destined to disappear later except one running towards the region of the papilla neglecta. In no less than a quarter of the ears examined, but usually on one side only in any animal, aberrant fibres could be seen leaving the superior ampullary nerve to invade mesenchyme outside the otic capsule. Streeter (1907) also observed of the posterior ampullary nerve in human embryos that, whereas later it formed a single trunk, at the 11 mm. stage the fibres were spread out into several separate bundles, possibly recapitulating a more primitive type of innervation. Single nerve fibres which failed to make contact with the epithelium of the utricular macula ('fibres égarées') were described in the human embryo by Tschernjachiwsky (1929). They appeared to lose themselves in the lumen of the utricle.

MATERIAL

The present investigation has been undertaken on serially sectioned human embryos of the London Hospital Anatomy Department collection prepared according to de Castro's silver impregnation technique.

The following embryos have been examined:

No. H. 214. C.R. length 15·5 mm., plane of section transverse.
No. H. 191. C.R. length 17·5 mm., plane of section transverse.
No. H. 180. C.R. length 30 mm., plane of section transverse.
No. H. 206. C.R. length 43 mm., plane of section coronal left half of head.
No. H. 206. C.R. length 43 mm., plane of section sagittal right half of head.
No. H. 218. C.R. length 48 mm., plane of section coronal left half of head.
No. H. 218. C.R. length 48 mm., plane of section sagittal right half of head.
No. H. 209. C.R. length 62 mm., plane of section coronal.
No. H. 125. C.R. length 92 mm., plane of section coronal left half of head.
No. H. 125. C.R. length 92 mm., plane of section sagittal right half of head.

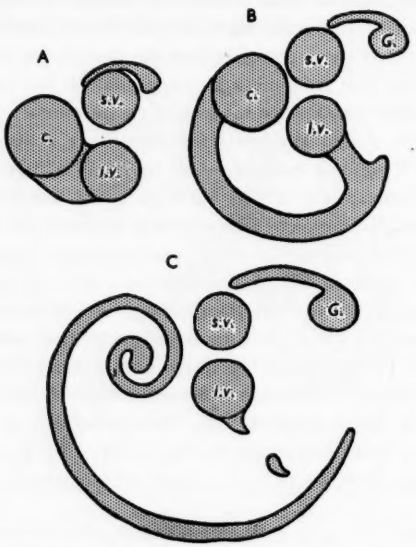
OBSERVATIONS

The acoustico-facial ganglion

In the 15 mm. embryo the acoustico-facial ganglion occupies completely the interval between the otic vesicle and the hind-brain. Its cranial end extends medially as far as the brain surface, and caudally it reaches the medial aspect of the budding cochlear duct. The geniculate ganglion cells form a definite lateral group lying

ventral to the motor root of the facial nerve. A column of facial cells extends centrally to the ventral aspect of the acoustic ganglion, and from there accompanies the sensory root of the facial nerve to its point of entry into the hind-brain.

By the 17·5 mm. stage the acoustic ganglion has migrated caudo-laterally, the interval between it and the hind-brain being bridged by fibres of the auditory nerve. Although the various parts of the ganglion are in continuity, three main groups of cells can be distinguished: two lateral, of which the more cranio-ventral forms the superior vestibular ganglion, and the more caudo-dorsal the inferior vestibular



Text-fig. 2. Schema showing three stages in the differentiation of the acoustico-facial ganglion into its geniculate (*G.*), superior vestibular (*S.V.*), inferior vestibular (*I.V.*) and cochlear (*C.*) parts, the last-named being most medial. A, 15–17·5 mm.; B, 30 mm.; C, 43–62 mm. The original vestibulo-cochlear column connecting the inferior vestibular and cochlear ganglia breaks down to leave an intermediate island of ganglion cells.

ganglion; and a medial, the cochlear ganglion, which fuses with the inferior vestibular group of cells caudally, to form a combined ganglionic mass closely encircled by the cochlear duct which is now making its first turn. The facial ganglion cells also show a further peripheral migration; they are no longer found on the course of the sensory root of the facial nerve central to the point where it hooks round the ventral aspect of the superior vestibular ganglion.

This disposition of ganglion cells is shown as stage A in Text-fig. 2.

The 30 mm. embryo is remarkable for the migration of cochlear ganglion cells which has occurred towards the basal turn of the cochlear duct. The main column of the cochlear ganglion traverses the cochlear spiral to come into apposition with its newly formed second turn. With the increase in diameter of the base of the spiral, the ganglion cells have maintained their close relation to the neuro-epithelium by a corresponding outward migration. Thus they form a somewhat thinned out ring

between the main cochlear group and the site of its original fusion with the inferior vestibular ganglion. The vestibulo-cochlear connexion appears at this stage as a column of cells migrating outwards from the caudal end of the inferior vestibular ganglion towards a point on the basal turn of the cochlear duct a little beyond its commencement.

The vestibulo-cochlear ganglionic column of the right ear appears in Pl. 1, fig. 4, and the general arrangement of the acoustico-facial ganglion at 30 mm. is shown diagrammatically as stage B in Text-fig. 2.

In the 43 mm. embryo the cochlear ganglion has followed the spiral growth of the cochlear duct (Pl. 1, fig. 3). Consequent upon further migration of ganglion cells to the basal turn, the vestibulo-cochlear column has now broken down, leaving an island of intermediate bipolar cells as a relic of the original fusion (Pl. 1, fig. 1). The 48 mm. embryo shows the cochlear ganglion separated from the inferior vestibular ganglion, but the elongated tip of the latter and the absence of intermediate cells suggest that further migration has still to occur. At the 62 mm. stage the cochlear duct is fully coiled, and the whole of the cochlear ganglion has become attenuated with the widening of the diameter of its spiral turns. The cochlear ganglion cells now occupy their definitive position in relation to the primordial organ of Corti, except for the intermediate island which is again present. The general arrangement is illustrated as stage C of Text-fig. 2.

By the 92 mm. stage there are no longer any intermediate ganglion cells, and the cochlear migration is complete. On the other hand, a thin column of facial ganglionic cells still extends centrally from the geniculate ganglion to the cranio-ventral aspect of the superior vestibular ganglion. The fully migrated cochlear cells undergo a maturation process whereby their cytoplasm is reduced in amount and the neurofibrils become more evident. The acoustic ganglion appears to consist exclusively of bipolar cells, with no evidence of ganglion cells of motor type.

The vestibulo-cochlear anastomosis of Oort

The central processes of the intermediate ganglion cells described above form a bundle which can be followed to the lateral aspect of the caudal end of the inferior vestibular ganglion. Since there is no other connexion between the inferior vestibular and cochlear ganglia, once an intermediate island has replaced the original vestibulo-cochlear column, this bundle can be identified with the anastomosis of Oort. It appears in the 43 and 62 mm. embryos as a collection of nerve fibres with a relatively poor silver affinity, which cannot be traced through the compact inferior vestibular ganglion (Pl. 1, figs. 1, 2). At the 92 mm. stage on the left side the fibres of the anastomotic bundle have stained darker and can be seen traversing the inferior vestibular ganglion whose cells are now more dispersed. This incorporation of Oort's nerve appears to be due to the further growth of the caudal end of the inferior vestibular ganglion from whose cells springs the nerve to the ampulla of the posterior semicircular duct. The right ear of the 92 mm. embryo does not show a definite vestibulo-cochlear bundle.

When migration of cochlear cells is complete, Oort's anastomosis normally comes to join the basal coil of the cochlear ganglion. The 43 mm. embryo, however, exhibits on the left side a curious anomaly. In addition to the main anastomosis

derived from the intermediate ganglion cells, there is also a second bundle leaving the lateral aspect of the inferior vestibular ganglion which can be traced peripherally to the part of the cochlear ganglion related to the middle turn of the cochlear duct. This aberrant anastomosis of Oort is presumably the result of an abnormality of the original cochlear migration (Pl. 1, figs. 2, 3).

The cochleo-saccular nerve of Hardy

In the 30 mm. embryo the saccular macula, recognizable by the presence of an intra-epithelial nerve plexus, at its caudal end is continuous dorsally with the more heaped-up sensory epithelium of the commencement of the cochlear duct. This junctional region is connected with the inferior vestibular ganglion by a well-stained compact nerve bundle running a direct course immediately cranial to the vestibulo-cochlear ganglionic column (Pl. 1, fig. 5). At the 43 mm. stage and after, the equivalent bundle coming off the inferior vestibular ganglion is drawn down with the developing anastomosis of Oort towards the basal turn of the cochlear duct. From there it takes a recurrent course to the caudal end of the saccular macula (Pl. 1, fig. 6). Since the macula receives no innervation from cochlear ganglion cells, the peripheral part of this bundle can be identified with the nerve described by Hardy, and the whole with that found by Rasmussen in his animal micro-dissections. The name 'cochleo-saccular nerve' may be retained, but must be understood to indicate its loop down to the basal cochlear turn, not a derivation from cochlear ganglion cells.

Pl. 2, fig. 1, shows in the 43 mm. embryo the inferior vestibular ganglion giving off its cochleo-saccular branch. In this case some fibres leave it to travel direct to the macula with the main saccular trunk. When the same nerve is traced peripherally, a few fibres seem to run towards the commencement of the cochlear duct. Later embryos, however, do not show any innervation of the organ of Corti by the cochleo-saccular nerve. Indeed, with increasing growth it becomes much attenuated, and even to the saccular macula its contribution is insignificant.

As Hardy pointed out, the cochleo-saccular nerve is not constantly present. In this series it is lacking in the 48 mm. embryo on both sides and in the 92 mm. embryo on the left.

The superior saccular nerve of Voit and a superior utricular nerve

Pl. 2. fig. 2, shows in the 48 mm. embryo an anastomotic bundle derived from the inferior vestibular ganglion running up to join the caudal aspect of the main nerve to the utricle. At the same time, as the utricular nerve winds round the cranial end of the saccule, a few of its caudalmost fibres leave the main trunk to supply the adjacent part of the saccular macula. This is the superior saccular nerve of Voit. It is partly continuous with the anastomotic bundle, so that some, though probably not all, of its cells of origin are located in the inferior vestibular ganglion.

The close apposition of the developing utricular nerve to the cranial end of the saccular macula is even more evident in the earlier embryos. Moreover, the common ampullary nerve in its course to the cristae of the superior and lateral semicircular ducts runs ventral to the utricle in close relation to the macula. As it does so, it gives off a few fibres to supply the neuro-epithelium. There is thus a superior utricular nerve providing an additional innervation to the ventral end of the utricular

macula quite comparable to that of the superior saccular nerve of Voit to the cranial end of the saccular macula. This superior utricular nerve is well seen at the 48 mm. stage (Pl. 2, fig. 5), and in the 30 mm. embryo appears together with Voit's nerve in a single section (Pl. 2, fig. 4).

Aberrant fibres of the ampullary nerves

Two types of variation are commonly found in the auditory complex: single fibres which fail to reach the neuro-epithelium, and bundles of fibres which may either lose their way likewise, or else reach their destination by an abnormal and devious route. As an example of the first type, a 'fibre égarée' in the 43 mm. embryo enters the lumen of the cochlear duct, thereby resembling those described by Tschernjachiwsky in relation to the utricle. Aberrant bundles are most commonly derived from those nerves which have the longest course to run through the otic capsule, that is, the nerves to the ampullary cristae.

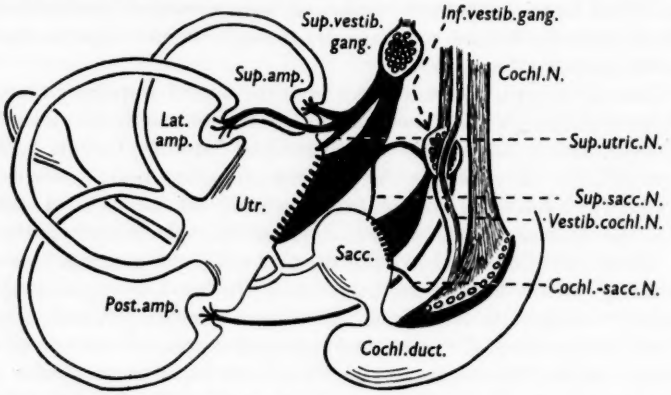
In the 30 mm. embryo, aberrant fibres from the lateral ampullary nerve on the left side pass out through the otic capsule to lose themselves in the mesenchyme of the middle ear. This is comparable to the condition found by Lorente de Nó to be so frequent in mice. At all stages the posterior ampullary nerve tends to give off small bundles which run through separate channels to reach the crista. An interesting anomaly of the superior ampullary nerve is seen in the right ear only of the 48 mm. embryo. About half the fibres leave the superior vestibular ganglion as a separate bundle running outside the inner ear proper with the facial nerve, piercing the otic capsule again to supply the lateral end of the superior crista (Pl. 2, figs. 5, 6). The nerve to the medial end of the crista runs a normal course. At the site of origin of the aberrant bundle the cranio-ventral tip of the superior vestibular ganglion extends up into the facial canal, close to the most centrally placed facial ganglion cells (Pl. 2, fig. 3).

DISCUSSION

From an early stage, ganglion cells extend from the main geniculate group centrally along the course of the sensory root of the facial nerve. This is consistent with the view that the facial ganglion develops originally from the neural crest cranio-medial to the acoustic ganglion proper, which separates off from the otic vesicle. The geniculate ganglion cells subsequently migrate laterally round the ventral aspect of the cranial end of the acoustic ganglion. They leave, however, a wake of ganglion cells, which persists as a column connecting the geniculate ganglion with the region of the tip of the superior vestibular ganglion. From here a few fibres often descend medial to the vestibular ganglion to join the cochlear nerve, corresponding exactly to a facio-cochlear anastomosis described by Lorente de Nó (1926) in mice. They are probably aberrant peripheral processes of the most central facial ganglion cells.

The cochlear ganglion originally appears as a compact column between the vestibular cells and hind-brain. It begins to separate off from the rest of the ganglion at its cranial end, i.e. that part from which the central processes emerge. The periphery of the caudal end of the column, which is still fused with the inferior vestibular ganglion, is in contact with the developing cochlear duct. From the first, therefore, there is a close relation between cochlear ganglion cells and cochlear

neuro-epithelium. The spiral of the cochlear ganglion grows *pari passu* with that of the cochlear duct, with a consequent thinning out of the ganglionic coil which occurs progressively from base to apex. As the ganglion cells achieve their definitive position in relation to the developing organ of Corti they undergo a process of maturation, becoming smaller in size. The latest cells to migrate are those which form a remnant of the original vestibulo-cochlear anastomosis. They eventually take up their position at a point on the basal turn of the cochlear duct some distance from the ductus reuniens, where they become indistinguishable from other cochlear ganglion cells. Their central processes, as a result of further caudal growth of the posterior ampullary nerve cells, come to traverse the inferior vestibular ganglion—hence Oort's vestibulo-cochlear anastomosis.



Text-fig. 3. Diagram of the right auditory complex, modified in accordance with the present findings. Oort's nerve is shaded to indicate its formation from central processes of cochlear ganglion cells. Hardy's nerve is a peripheral branch of the inferior vestibular ganglion. The latter also sends a contribution to the main utricular nerve, some of which may pass into the nerve of Voit. A superior utricular nerve comes off the common ampullary nerve.

It is extremely difficult to follow with certainty and for any distance the course of single nerve fibres derived from any particular bipolar cell. Consequently, it is only by virtue of the constant association that has been noted between the vestibulo-cochlear bundle and an island of 'intermediate' ganglion cells present at a certain stage of development that it is possible to confirm Lorente de Nó's supposition that Oort's anastomosis is of cochlear origin. It is misleading, however, to describe the bundle as consisting of 'aberrant' cochlear fibres, since it appears to be of normal occurrence and explicable in terms of normal development.

Although the presence of central processes of cochlear ganglion cells in Oort's anastomosis has been demonstrated, the possibility has not been excluded that it may also contain nerve fibres of other types. If any fibres of vestibular origin are present, it is probable that they are truly aberrant and fail to make contact with the organ of Corti: since it has been shown that the cochleo-saccular nerve, which is certainly vestibular and runs in close association with the vestibulo-cochlear bundle, does not do so. The experimental results of G. L. Rasmussen are highly suggestive that efferent fibres of some sort run with the anastomosis, although it is not true to

assert that it is a purely motor nerve, at least in man. If, in fact, parasympathetic secretomotor fibres serve the inner ear, it is certainly possible that they approach it by means of the more primitive vestibular root of the auditory nerve, eventually reaching the cochlea, which comes to be the main site of endolymph secretion, along the scaffolding of the vestibulo-cochlear anastomosis. But the problem remains, if there are preganglionic fibres, where are the post-ganglionic cells?

Autonomic fibres of sympathetic type are found. They accompany the blood vessels of the labyrinth, and can be seen especially in the later embryos.

Since the cochleo-saccular nerve has been shown to be of vestibular origin, and since there is no evidence of the presence of effective vestibular fibres in the vestibulo-cochlear nerve, it follows that there is no common innervation of saccule and cochlea, and thus there is demolished one anatomical prop for the argument that the saccule functions as an organ of hearing.

On the other hand, the separation of the vestibular ganglia into superior and inferior divisions appears to be of no functional importance. Not only is the posterior crista (as is well known) represented in the inferior vestibular ganglion, but also probably the dorsal end of the macula of the utricle, through the communication from the ganglion to the utricular nerve. The significance of a separate innervation to the cranial end of the saccular macula by Voit's nerve is diminished by the finding that the cranio-ventral end of the utricular macula constantly acquires a twig from the common ampullary nerve in a very similar fashion. Presumably in each case the neuro-epithelium is sufficiently developed to exert a neurotropic attraction on a few fibres of the nerve bundle growing out past it. By contrast, although the nerve to the posterior crista can be seen in early embryos to run immediately deep to the epithelium of the medial wall of the saccule, the saccular macula is too immature at the time of its outgrowth ever to attract any fibres from it.

By a curious error a number of published diagrams of the labyrinth show the posterior ampullary nerve running *lateral* to the saccule.

Although in the distribution of the auditory nerve deviations from the normal have been so little noticed in the past, they seem to be common. It is possible that single fibres may go astray as a result of some peculiarity of the ground substance through which they grow, whereas in the case of an aberrant nerve bundle the cause may lie in an abnormal proliferation of the parent ganglion cells. Thus in the embryo where half the innervation of one superior crista reaches it by an anomalous route, it appears that the exuberant growth of the superior vestibular ganglion has caused its cells to spread up into the facial canal. In the case of the abnormal anastomosis of Oort, some ganglion cells of the vestibulo-cochlear junctional region may in the course of development have come under the influence of the neuro-epithelium of the middle turn of the cochlear duct.

In general, many puzzling features of the auditory complex, normal and abnormal, are best interpreted ontogenetically, in terms of the way in which the subdivisions of the acoustico-facial ganglion grow out from one another. The standard sensory innervation pattern found in the present series of embryos is illustrated by Text-fig. 3. Apart from the slender facio-cochlear strand already noted, there was no evidence of cranial nerves other than the eighth contributing to the supply of the inner ear.

SUMMARY

1. The development of the innervation of the otic labyrinth has been studied in human embryos stained by de Castro's silver impregnation technique.

2. Some of the complexities of the adult innervation can be related to the manner in which the acoustico-facial ganglion differentiates into its component parts.

3. There is no evidence of any innervation of the base of the cochlear duct from vestibular ganglion cells, nor of the saccule from cochlear ganglion cells.

4. The vestibulo-cochlear anastomosis of Oort (1918), which has been regarded as of vestibular origin, is formed by central processes of the latest group of ganglion ›igrate to the cochlea. It is a relic of the original fusion of the inferior vestib.. .nd cochlear ganglia.

›chleo-saccular nerve of Hardy (1934), which has been regarded as of cochlear origin, is a branch of the inferior vestibular ganglion.

6. The superior saccular nerve of Voit (1907) has a counterpart in a branch of the common ampullary nerve innervating the utricle.

7. Anomalies of the innervation occur. Aberrant sensory fibres of the seventh cranial nerve may run peripherally with the cochlear nerve. There is no true facio-vestibular anastomosis.

This paper has been made possible by the generosity of Prof. J. D. Boyd in giving me access to his unique collection of human embryos. I wish to thank him and Prof. E. W. Walls for their unfailing encouragement and advice, and also Mr R. Quinton Cox, A.I.M.L.T., of the Anatomy Department, London Hospital Medical College, who took the photographs.

REFERENCES

Buskirk, C. van (1945). The seventh nerve complex. *J. comp. Neurol.* **81**, 169–191.

Hardy, M. (1934). Observations on the innervation of the macula sacculi in man. *Anat. Rec.* **59**, 403–418.

Kappers, C. U. Ariëns, Huber, C. & Crosby, E. (1936). *The Comparative Anatomy of the Nervous System of Vertebrates, including Man*, **1**, 484–502. New York: The Macmillan Co.

Lorente de Nó, R. (1926). Études sur l'anatomie et la physiologie du labyrinthe de l'oreille et du VIII[e] nerf. *Trab. Lab. Invest. biol. Univ. Madr.* **24**, 53–153.

Lorente de Nó, R. (1933). Anatomy of the eighth nerve. *Laryngoscope, Lond.*, **43**, 1–38.

Oort, H. (1918). Über die Verästelung des Nervus octavus bei Säugetieren. *Anat. Anz.* **51**, 272–280.

Pearson, A. A. (1947). The roots of the facial nerve in human embryos and foetuses. *J. comp. Neurol.* **87**, 139–159.

Poljak, S. (1927). Über die doppelte Innervation der Macula sacculi und über das cochleo-vestibulare Bundel bei den Säugetieren. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **84**, 145–152.

Rasmussen, G. L. (1946). The olivary peduncle and other fibre projections of the superior olivary complex. *J. comp. Neurol.* **84**, 141–219.

Streeter, G. L. (1907). On the development of the membranous labyrinth and the acoustic and facial nerves in the human embryo. *Amer. J. Anat.* **6**, 139–165.

Streeter, G. L. (1912). In *Manual of Human Embryology*, **2**, 129–132. By Keibel, F. & Mall, F. P. Philadelphia and London: J. B. Lippincott Co.

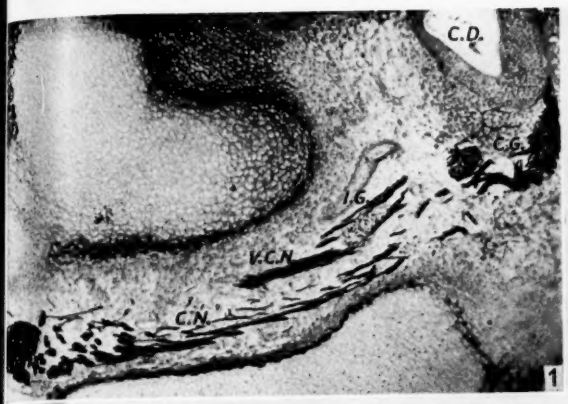
Trampusch, H. A. L. (1942). On ear-induction. *Acta neerland.* **4**, 195–213.

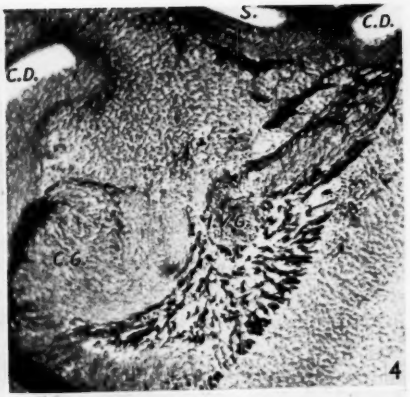
Tschernjachiwsky, A. (1929). Sur les fibres nerveuses égarées dans l'embryon humain. *Trab. Lab. Invest. biol. Univ. Madr.* **26**, 99–105.

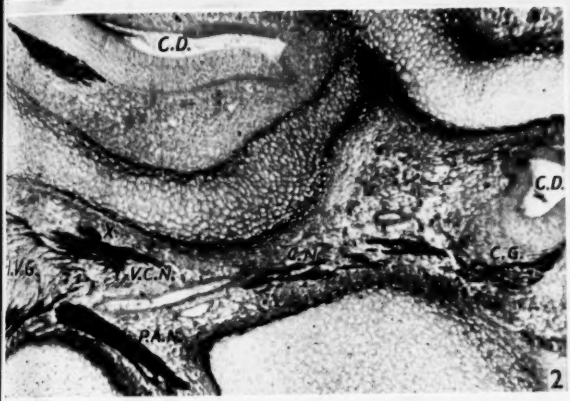
Voit, M. (1907). Sur Frage der Verästelung des Nervus acusticus bei den Säugetieren. *Anat. Anz.* **31**, 635–640.

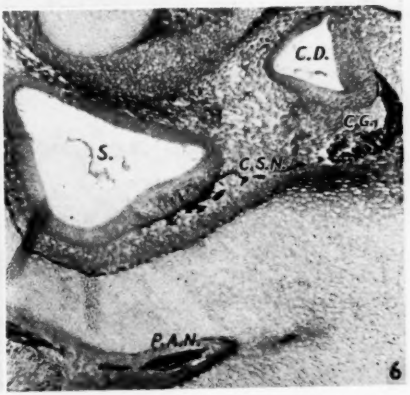
Weston, J. (1937). Observations on the distribution of ganglion cells and fibres related to the saccule and the basal coil of the cochlea. *Acta neerland.* **1**, 136–150.

Weston, J. (1939). Notes on the comparative anatomy of the ganglion cells associated with the vertebrate inner ear sensory areas. *J. Anat., Lond.*, **73**, 263–288.

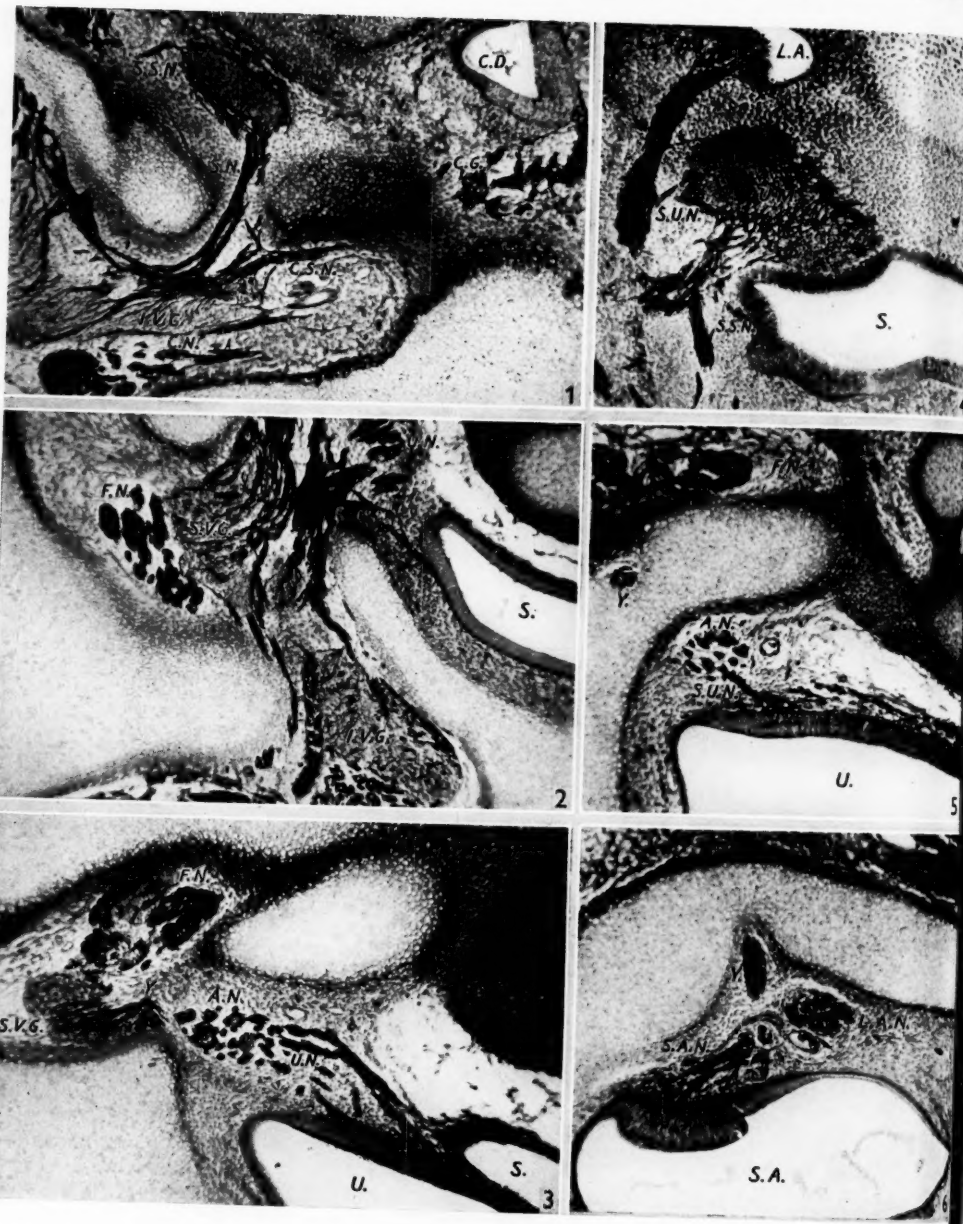








SHUTE—NERVOUS PATHWAYS IN THE DEVELOPING HUMAN LABYRINTH



SHUTE—NERVOUS PATHWAYS IN THE DEVELOPING HUMAN LABYRINTH

EXPLANATION OF PLATES

All photomicrographs × 100.

Abbreviations.

A.N.	common ampullary nerve	*S.A.*	superior ampulla
C.D.	cochlear duct	*S.A.N.*	superior ampullary nerve
C.G.	cochlear ganglion	*S.N.*	saccular nerve
C.N.	cochlear nerve	*S.S.N.*	superior saccular nerve
C.S.N.	cochleo-saccular nerve	*S.U.N.*	superior utricular nerve
F.N.	facial nerve	*S.V.G.*	superior vestibular ganglion
I.G.	intermediate ganglion cells	*U.*	utricle
I.V.G.	inferior vestibular ganglion	*U.M.*	utricular macula
L.A.	lateral ampulla	*U.N.*	utricular nerve
L.A.N.	lateral ampullary nerve	*V.C.N.*	vestibulo-cochlear nerve
P.A.N.	posterior ampullary nerve	*X.*	aberrant fibres of vestibulo-cochlear nerve
S.	saccule	*Y.*	aberrant fibres of superior ampullary nerve

Plate 1

Fig. 1. Left ear of 43 mm. embryo, coronal section. To show the intermediate ganglion cells and their central processes forming the vestibulo-cochlear nerve of Oort.

Fig. 2. Right ear of 43 mm. embryo, sagittal section. To show the vestibulo-cochlear nerve in relation to the inferior vestibular ganglion. An aberrant vestibulo-cochlear bundle is also present, cut transversely.

Fig. 3. The same aberrant vestibulo-cochlear bundle traced to its origin from cells of the middle coil of the cochlear ganglion.

Fig. 4. Right ear of 30 mm. embryo, transverse section. To show the inferior vestibular and cochlear ganglia still connected by a vestibulo-cochlear column of cells. This corresponds to stage B of Text-fig. 2.

Fig. 5. In the same ear, the cochleo-saccular nerve is seen running from the inferior vestibular ganglion to the region where the saccule joins the commencement of the cochlear duct.

Fig. 6. Left ear of 43 mm. embryo, coronal section. The peripheral portion of the cochleo-saccular nerve, running from the region of the cochlear ganglion to the caudal end of the saccular macula, is that described by Hardy.

Plate 2

Fig. 1. The cochleo-saccular nerve, as seen in Pl. 1, fig. 6, but traced to its origin from the inferior vestibular ganglion. Some fibres leave its trunk to run direct to the saccule with the main saccular nerve. The superior saccular nerve of Voit is also shown.

Fig. 2. Left ear of 48 mm. embryo, coronal section. To show the contribution from the inferior vestibular ganglion to the utricular nerve, which is giving off the superior saccular nerve.

Fig. 3. Right ear of 48 mm. embryo, sagittal section. To show the central end of the column of ganglion cells which extends along the facial nerve from the geniculate ganglion. The superior vestibular ganglion is here seen spreading up into the facial canal and giving rise to an aberrant bundle destined for the lateral end of the superior crista.

Fig. 4. Right ear of 30 mm. embryo, transverse section. To show the utricular nerve giving off the superior saccular nerve as it winds round the saccule, and the common ampullary nerve giving off a superior utricular nerve as it winds round the utricle.

Fig. 5. Right ear of 48 mm. embryo, sagittal section. The superior utricular nerve is well shown. The aberrant fibres to the superior crista seen in Pl. 2, fig. 3, are still extracapsular.

Fig. 6. The aberrant superior cristal fibres of the previous figure have reentered the otic capsule. The medial end of the superior crista is being supplied by fibres which have run a normal course with the common ampullary nerve.

APLASIA OF THE MALE PREPUCE WITH EVIDENCE OF HEREDITARY TRANSMISSION

By THEODORE JAMES
Duchess of York Hospital for Babies, Manchester

The condition of aplasia of the male prepuce is one to which I have been unable to find any reference in the literature, and unless knowledge of the possibility of such a condition and interest in its frequency provoke inquiry, it seems that the condition may continue to pass unrecognized. I am led to this opinion by the way in which I became aware of the possibility of a boy being born circumcised, that is, with aplasia of the prepuce.

I came across my first case in 1947. In 1950 I met with another and in 1951 I have seen two more.

Case I. In 1947 I was asked to see a boy 6 years of age on account of a minor complaint. At the time of examination he showed an unusual shyness when his genitalia were exposed. His mother, observing this, explained that his shyness was due to the fact that he had been made self-conscious at school where few, if any, of his schoolmates were circumcised, whereas her boy had been 'born circumcised', to use her own words. Without this information it would have been reasonable to assume that the child had been quite expertly circumcised by human hands.

Case II. At the age of 3 months, this male infant was brought to the out-patient department by his mother, for advice about a meatal ulcer. When it was remarked that this was the occasional price one had to pay for circumcision, the mother said, with friendly asperity, that her son had not been circumcised but 'took after his father', and so the fact that her son had been born without a foreskin occasioned no surprise in the family. The father knew from his parents that he had been born without a foreskin. Pl. 1, fig. 1, shows the natural absence of this infant's prepuce and the meatal ulcer.

Case III. This case came to my attention about 9 months after case II. A mother brought her infant son, aged 6 months, to the out-patient department because of three small ulcers on the glans penis, one being at the meatus. On this occasion I remarked that the circumcision had not been as complete as it might have been, there being some slight (apparent) redundancy of preputial tissue which had become chafed by the diaper. It was suggested that the circumcision should be repeated to improve the appearance of the penis. This brought forth the protestation from the mother that her baby had never been circumcised and that 'he has been so since he was born'. Pl. 1, fig. 2, shows nature's abortive attempt to form a prepuce.

Case IV. This example, surprisingly enough, was seen in the out-patient department the day after case III had been observed. A young and inexperienced mother sought advice about a feeding formula for her 6-week-old boy and requested also a routine physical examination. At the time of the examination, the perfect

circumcision of the prepuce was commented upon, and the mother was warned against the threat of a meatal ulcer developing. She, smiling, assured me that her baby had not been circumcised, and in all her innocent ignorance 'thought that all baby boys were born like that'. On request, she was able to give some additional information at a later date. The paternal grandfather and his son (the baby's father) were both completely devoid of prepuces and yet had never been circumcised. The grandfather prefaced his remarks by saying that neither he nor his son 'ever had need of it'. Pl. 1, fig. 3, illustrates the aplasia in this case.

COMMENT

It may be of interest to recapitulate briefly the accepted theory of the development of the male prepuce, based essentially on the work of Hunter (1935). In the beginning the glans penis is quite naked and is marked off from the shaft by a shallow groove. About the 70 mm. stage growth of the cavernous tissue appears to lag behind that of the ectodermal tissue, which reduplicates itself and appears to flow over the glans, becoming recognizable as the beginning of the prepuce. The urethral opening at this stage is placed well back on the ventral surface of the penis. By the 170 mm. stage the prepuce completely encloses the glans, which is marked along its ventral surface by a groove. The lips of this groove then fuse together to form the glans portion of the urethra, and with the folds of the advancing prepuce to form the frenulum.

In the earlier 45 mm. stage the male genitalia approximate to their final form (Hamilton, Boyd & Mossman, 1947), and by the time the primitive urogenital orifice is well forward on the under-surface of the glans, the coronary sulcus or groove, separating the glans from the shaft of the penis, has appeared. The external urethral orifice has moved progressively from behind forwards by the fusion of the edges of the urethral folds on the ventral surface. It is to these two distinct embryologic stages in the development of the phallus that two anatomic facts can be attributed. The one is that where there is aplasia of the prepuce, there is no abnormality of the urethra or its opening; the second, that where there is a moderate or severe degree of hypospadias due to a faulty arrest of the progressive fusion of the primitive urethral folds, there results a malformed or 'hooded' prepuce.

Hamilton *et al.* (1947) mention double or bifid penis, hypospadias, perineal urethral fistula or complete hypospadias, epispadias associated or not with ectopia vesicae, among the abnormalities of the male external genitalia, but make no mention of an aplasia of the prepuce.

The four cases of failure of development of the male prepuce which have come under my observation, together with a hereditary background definitely established in two and unknown in the other two, lead me to believe that the condition cannot be an uncommon one. In this connexion Prof. J. M. Yoffey of Bristol has very kindly made available to me information that the condition was recognized in ancient times and 'is referred to in the Talmud where, in the Tractate "Sabbath", there is a reference to a dispute between the School of Shammai and the School of Hillel concerning a child who was born circumcised, and the time of whose circumcision would normally have fallen upon the Sabbath. From this dispute it is quite clear that the not infrequent existence of such children was a recognized occurrence

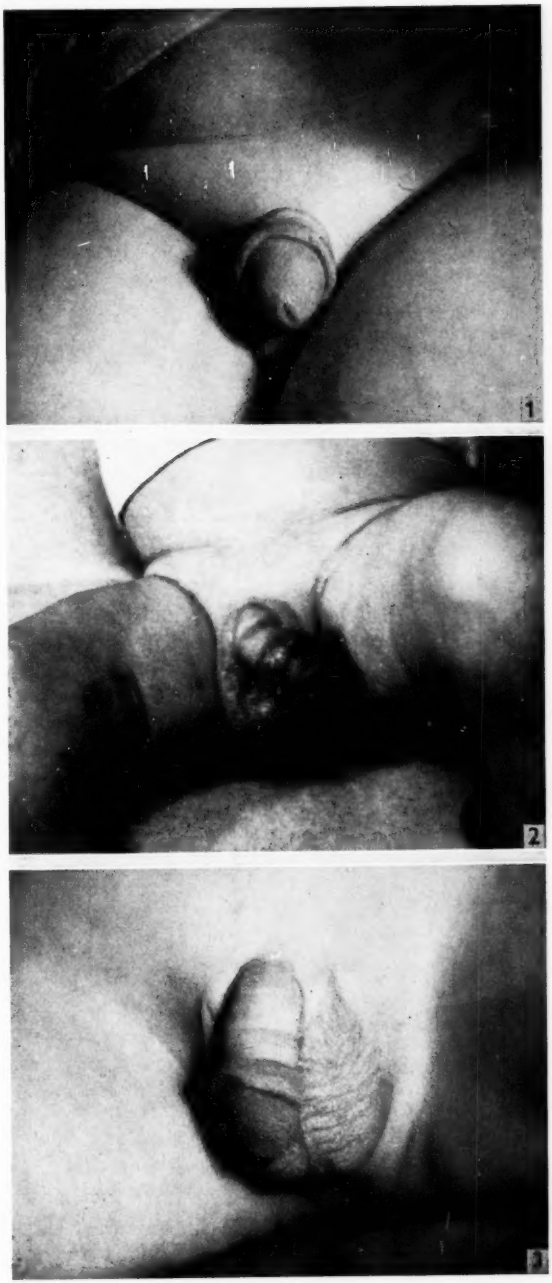
which was taken for granted.' Some of the infants and children seen for the first time in paediatric practice and assumed to have been circumcised actually may not have been so treated.

REFERENCES

HAMILTON, W. J., BOYD, J. D. & MOSSMAN, H. W. (1947). *Human Embryology.* Baltimore: The Williams and Wilkins Co.

HUNTER, R. H. (1935). Notes on the development of the prepuce. *J. Anat., Lond.*, **70**, 68–95.

Talmud: Tractate 'Sabbath', p. 135*a*.



JAMES—APLASIA OF THE MALE PREPUCE

QUANTITATIVE ASPECTS OF THE GROWTH OF THE HUMAN OVUM AND FOLLICLE

By S. H. GREEN and S. ZUCKERMAN,
Department of Anatomy, University of Birmingham

The development of the mammalian follicle relative to that of the oocyte can be divided into two distinct phases, the regression line relating ovular to follicular size being steep in the first phase, and practically horizontal in the second. The point at which the two lines intersect gives the approximate time, in relation to follicular size, at which the oocyte completes its growth. Detailed information on this subject is available for the mouse (Brambell, 1928); the rat, ferret, rabbit and pig (Parkes, 1931); the baboon (Zuckerman & Parkes, 1932); the common shrew (Brambell, 1935); the lesser shrew (Brambell & Hall, 1936); the rhinolophid bats, *Rhinolophus hipposideros minutus* and *R. ferrum-equinum insulanus* (Matthews, 1937); and the rhesus macaque, *Macacus mulatta* (Green & Zuckerman, 1947).

The purpose of the present note is to provide corresponding information for the human oocyte and follicle.

MATERIAL AND METHODS

Serial sections of eighty ovaries were examined, but few provided any information of value because (1) some of the material had been gathered in post-mortem rooms, and degeneration had occurred in the interval between death and autopsy; or (2) even when the interval was not unduly prolonged, the routine methods used in pathological laboratories are usually inadequate for the fixation of an organ as large as the human ovary.

Nevertheless, since, in spite of widespread inquiry, we have failed to obtain for examination adult human ovaries in a state comparable with those of experimental animals, we have thought it worth while to assemble and analyse such data as we have managed to collect. It is hoped that the publication of the present results will make it possible for them to be improved upon by workers in a better position than we to obtain normal human ovaries for examination.

The methods of measurements were the same as those used in our previous study (Green & Zuckerman, 1947). Taking a continuous series of sections, a search was made under the microscope for measurable follicles. The section giving the largest dimensions of each follicle was selected, and the oocyte and follicle measured in two directions at right-angles to each other on tracings of projected images.

RESULTS

Ninety-one observations relating to the first stage of growth were obtained from measurements of a single ovary of a child of 6 years. Since most large follicles and ova were undergoing degeneration in the specimens available to us, only a few observations relating to the second phase of follicular growth could be obtained from a single specimen. In all, we managed to collect thirty-three observations relating to this phase of growth from thirteen ovaries.

Regression equations in the form of $y=a+bx$, where y is the diameter of the oocyte and x that of the follicle, were calculated for the two phases of growth after the data had been plotted so as to eliminate those measurements which fell in the region of intersection of the two regression lines. The values obtained were as follows:

Phase I : $a=+10{\cdot}28\pm1{\cdot}45$: $b=+0{\cdot}59\pm0{\cdot}03$;
Phase II : $a=+75{\cdot}11\pm3{\cdot}09$: $b=+0{\cdot}001\pm0{\cdot}0015$.

The 'best-fit' lines are shown in Fig. 1.

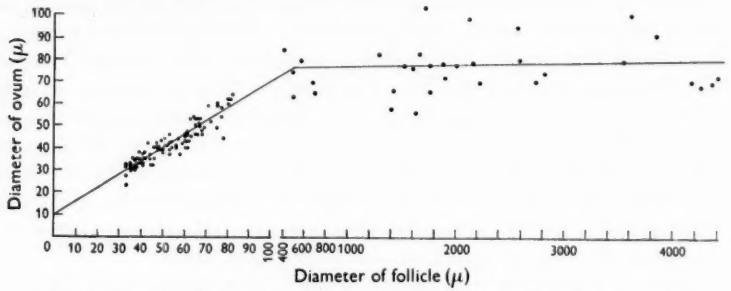


Fig. 1. Regression lines relating size of ovum and follicle in fixed human ovaries.

DISCUSSION

The present data indicate that in its quantitative aspects, the growth of the human ovum and follicle follows the same course as in other placental mammals. Furthermore, the value of b (0·59), as determined in the present study for the first phase of growth of the human ovum, does not differ markedly from that in some other species (e.g. the rhinolophid bat: $b=0{\cdot}60$; ferret: $b=0{\cdot}58$). It should be remembered, however, that differences in the slope of the best-fit line for the first phase of follicular growth may be statistically significant not only between species but also between individuals within a species.

As already observed, the point of intersection of the best-fit line relating the size of oocyte to follicle in the two phases of follicular growth gives an indication of the size at which the oocyte completes its growth. The value indicated by the present data (based on fixed and sectioned material) is 110 μ. It is obvious, however, that the point of intersection is very sensitive to slight differences in the value of a and b. Its 5 % fiducial limits for the present data are approximately 90 and 140 μ.

This range is smaller than that of 120–190 mm., which Hamilton (1944) gives for fresh human ova which various workers, including himself, have recovered from the uterine tube. The difference between the two sets of figures is probably due to shrinkage during fixation. In Hamilton's experience this may account for a decrease of as much as 20 %. Menkin & Rock's (1948) observations on two- or three-cell stage human embryos suggest that it may be as much as 50 %.

SUMMARY

The relationship of the growth of the ovum to follicle follows the same course in man as in other placental mammals in which the quantitative aspects of the process have been studied. The process may be divided into two phases. The regression line

relating ovular to follicular size is steep in the first phase, and practically horizontal in the second. The value of *b* for the first phase of follicular development is similar to that in other mammalian species.

We are much indebted to Prof. Dorothy Russell for the loan of a number of specimens under her care in the Pathological Department of the London Hospital.

REFERENCES

Brambell, F. W. R. (1928). The development and morphology of the gonads of the mouse. Part III. The growth of the follicles. *Proc. Roy. Soc.* B, **103**, 258–272.

Brambell, F. W. R. (1935). Reproduction in the common shrew (*Sorex araneus* Linnaeus): I. The oestrous cycle in the female. II. Seasonal changes in the reproductive organs of the male. *Philos. Trans.* B, **225**, 1–49.

Brambell, F. W. R. & Hall, K. (1936). Reproduction of the lesser shrew (*Sorex minutus* Linnaeus). *Proc. zool. Soc. Lond.* pp. 957–969.

Green, S. H. & Zuckerman, S. (1947). A comparison of the growth of the ovum and follicle in normal rhesus monkeys, and in monkeys treated with oestrogens and androgens. *J. Endocrinol.* **5**, 207–219.

Hamilton, W. J. (1944). Phases of maturation and fertilization in human ova. *J. Anat., Lond.*, **78**, 1–4.

Matthews, L. H. (1937). The female sexual cycle in the British horse-shoe bats, *Rhinolophus ferrum-equinum insulanus* Barrett-Hamilton, and *R. hipposideros minutus* Montagu. *Trans. zool. Soc. Lond.* **23**, 224–255.

Menkin, M. F. & Rock, J. (1948). *In vitro* fertilization and cleavage of human ovarian eggs. *Amer. J. Obstet. Gynaec.* **55**, 440–452.

Parkes, A. S. (1931). The reproductive processes of certain mammals. II. The size of the Graafian follicle at ovulation. *Proc. Roy. Soc.* B, **109**, 185–196.

Zuckerman, S. & Parkes, A. S. (1932). The menstrual cycle of the Primates. Part V. The cycle of the baboon. *Proc. zool. Soc. Lond.* pp. 139–191.

REINNERVATION OF STRIATED MUSCLE AFTER ACUTE ISCHAEMIA

By D. B. ALLBROOK and J. T. AITKEN
University College, London

Much has been written on the regenerative powers of mammalian striated muscle since it was described by Waldeyer in 1865. A review of the early work is given by Küttner & Landois (1913), and more recently by Fishback & Fishback (1932), Le Gros Clark (1946) and Harman (1947).

However, in these studies on the regeneration of muscle the authors have not described the changes that occur in the intramuscular nerves. Le Gros Clark states that muscle may proceed to functional recovery following acute ischaemia, but no report is given of the histology of the nerves, nor of the results of electrical stimulation of them.

Bowden & Gutmann (1949) have described the late changes seen in human cases of Volkmann's ischaemia, and note that there is neither effective reinnervation, nor effective muscle regeneration, due to the intense fibrosis. Little has been written on the early changes in the intramuscular nerves following acute ischaemia, and this investigation was undertaken with the following main objects in view:

(1) To study the early changes which occur in partially devascularized muscle, with special reference to its intramuscular nerves and motor end-plates.

(2) To investigate the method, rate and extent of reinnervation of devascularized muscle.

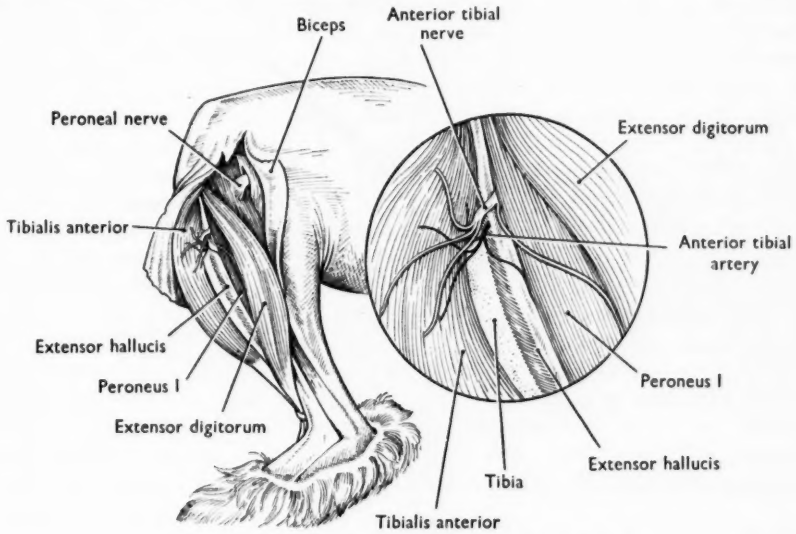
(3) To assess the importance of such factors as fibrous tissue deposition in the reorganization of the muscle.

EXPERIMENTAL METHODS

Adult rabbits were used throughout. Under nembutal and ether anaesthesia the anterior tibial artery and veins were divided between ligatures, care being taken not to damage the adjacent anterior tibial nerves. The vessels were approached by reflexion of the lateral free border of tibialis anterior and dissection in the space between extensor digitorum and extensor hallucis. The nerve passes superficial to the latter muscle and the blood vessels pass deep to it, or through its deeper part (Text-fig. 1). Any obvious subsidiary vessels passing into tibialis anterior were also divided. Thus the tibialis anterior muscle was deprived of its blood supply except for small vessels derived from the periosteum at the bony attachments. Immediately after operation the integrity of the muscle nerve was confirmed by electrical stimulation.

The degree of devascularization was demonstrated by the injection of 4% bromophenol blue into an ear vein. The solution was prepared by dissolving 1·0 g. of the dye in 25 ml. of water and adding 16 ml. of N/10-NaOH. This dye was shown by Rous & Gilding (1929) to demonstrate accurately the effectiveness of the circulation in muscle (Pl. 1, fig. 1).

In the long-term experiments, animals were given a further anaesthetic 3 days after devascularization and were reinjected with 4% bromophenol blue to demonstrate the extent to which revascularization had occurred. The muscles were stimulated electrically both directly and indirectly, and a strip of muscle from the lateral side of tibialis anterior was removed for histological examination. A final biopsy was conducted under anaesthetic at varying intervals up to 100 days after devascularization. The colour, size and consistency of the muscles were then observed, and they were stimulated directly and indirectly and the response noted. The stimulation consisted of rectangular pulses of 0·1 msec. length, at 1 sec. intervals, delivered from a multi-vibrator type stimulator.

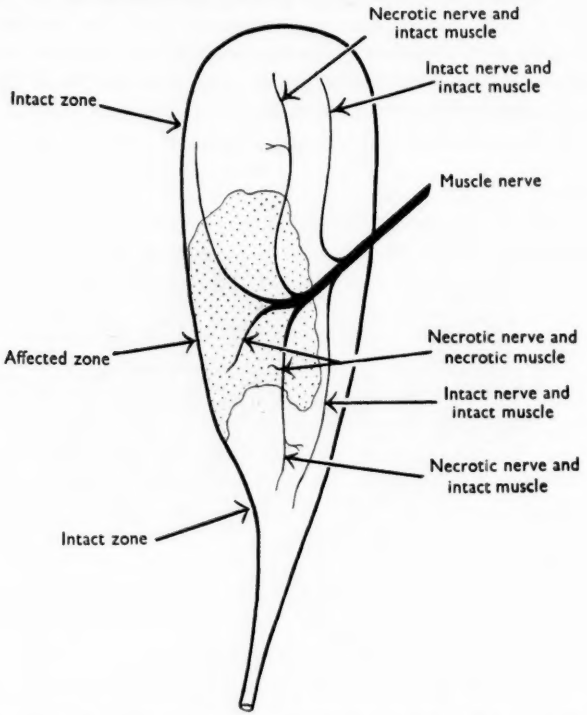


Text-fig. 1. Diagram illustrating the site of operation.

Muscle contractions were confirmed by separating the muscle from its neighbours and division of the distal tendon, to which was fixed a light pair of haemostatic forceps. In this way contraction of the tibialis anterior could be differentiated from that of surrounding muscles. That the response of the muscle was due to indirect stimulation and not to spread along the nerve was confirmed by the absence of the contraction on stimulation after crushing the nerve as it entered the muscle.

Muscles were then removed and fixed for a fortnight in 10% formalin with 2% pyridine in water. Sections, 50μ thick, were cut on the freezing microtome and stained by a modified Bielschowsky-Gros silver impregnation technique (Aitken, 1950). It was possible to differentiate normal and pathological muscle in suitably stained preparations. The necrotic tissues were very darkly stained compared with adjacent pale regenerating muscle. Variation in the quantity of ammonia in the ammoniacal silver solution and in the length of time that sections remained in this solution governed the intensity of staining of the cellular background. The speed at which the silver was deposited in the sections, whilst in the ammoniacal silver, could

be conveniently retarded by the addition of 4–6 ml. of absolute alcohol after the ammonia. A few muscles were embedded in paraffin and stained with haematoxylin and eosin. In some cases the muscle nerve was also removed at the final biopsy, fixed in Flemming's solution (Aitken, Sharman & Young, 1947), and transverse sections were cut at 5μ intervals and stained by Weigert's method.



Text-fig. 2. Diagram of tibialis anterior muscle showing the intact and affected zones. The intermediate zone is not shown. The distribution of the intramuscular branches of the nerve is outlined.

MUSCLE ZONES

Naked-eye examination of the muscles during the first 12 days shows well-defined zones of change as a result of the devascularization (Text-fig. 2).

Intact zones. At either end of the muscle variable lengths of muscle fibres are maintained in a healthy state by small blood vessels coming from the tibial periosteum and along the muscle tendon. Immediately after operation these zones are coloured an intense blue after intravenous bromophenol-blue injection. Histologically the muscle in this zone varies only slightly from normal at any period after operation.

Affected zones are usually found in the middle one-third or two-thirds of the muscle. These areas do not stain after bromophenol-blue injection, up to 3 days have defective circulation, and histologically show a complex cycle of changes.

Intermediate zones are situated between the intact zones and the affected zones.

Immediately after operation they are stained a faint blue. They have a poor circulation, and in the early days muscle and nerve appear to be damaged but not to the same extent as in the affected zones. At a later period these zones cannot be clearly distinguished from the normal and affected zones.

RESULTS

Eighteen hours after devascularization

There is no contraction on direct or indirect stimulation of the middle two-thirds of the muscle. The upper end can be seen to contract on direct stimulation only. The intact zones stain the same colour with bromophenol-blue injection as surrounding muscles, the intermediate zones are much paler, whilst in the affected zones the muscle remains quite unstained, and is sharply demarcated from the surrounding tissues. The muscle is swollen, friable, and light brown or yellowish in colour. Small haemorrhages and greyish patches are present over its surface. The surrounding fascia shows hyperaemia and oedema, and on cutting into the affected zone of the muscle a slow venous ooze occurs.

Histology of muscle fibres. Extensive changes have already appeared in the muscle after 18 hr. and the three zones can be readily defined. In the *affected zones* there is considerable separation of muscle fibres from each other, probably due to the accumulation of oedema fluid between the fibres. There is an infiltration between the fibres by polymorphonuclear leucocytes. The outline of the muscle fibres is blurred and they are wavy in shape, being alternately swollen and constricted. The transverse striations are frequently absent, and the sarcoplasm has a 'frosted-glass' appearance. Longitudinal fissuring appears and is similar to that reported by Barer (1947) after damage to muscle fibres. In the *intermediate zone* separation between fibres is less obvious. Some fibres retain their outline and transverse striations, while others show a wavy outline and 'frosted-glass' appearance. The number of infiltrating polymorphs is less than in the affected zone. The *intact zone* consists of apparently normal muscle fibres.

Histology of nerve 18 *hr. after devascularization.* In the *affected zone* there is segmentation of the axons into short lengths having a wavy outline, as described by Young (1942). In other cases particles of dark-staining debris occupy the neurilemmal tubes. In other cases the neurilemmal tubes seem to be empty except for necrotic Schwann cell nuclei (Pl. 1, fig. 2). The motor end-plates cannot be identified and already appear to have been completely destroyed.

The intramuscular nerves of the *intermediate zone* are variable in structure. A few axons and motor end-plates are intact, probably because they pass through a part of the muscle which has not been devascularized or only partially so, whilst many of the nerves show advanced segmentation and necrosis of the axons. Some motor end-plates are granular and the fine terminal branches are absent. A common finding is a breaking up of the motor end-plates and terminal few microns of the axon, the more proximal part of which remains intact (Pl. 1, fig. 3). We suggest that this is a transitory stage which soon results in a sprouting of the axon to give the appearance seen in Pl. 3, fig. 15.

In the *intact zones*, most nerve fibres appear to be undamaged, though occasionally a segmenting fibre can be seen.

Two days after devascularization

Indirect stimulation of the muscles resulted in sixteen out of twenty-two failing to contract. Eight out of twenty-two did not contract on direct stimulation. The macroscopic appearance is similar to that at 18 hr., except that many dilated blood vessels now pass into the muscle from the surrounding fascia. Injection of bromophenol blue showed a diminution in the area with defective blood supply, as compared with the area seen immediately after operation. As the tibialis anterior is dissected off the tibia, the dilated periosteal vessels bleed freely. In six out of thirteen muscles small blood vessels were seen running with the muscle nerve. The importance of the small blood vessels of the nerve in the nutrition of the muscles does not appear to be great, though their importance to the nerve is undoubted and they may account for the occasional presence of apparently intact nerves in the affected zones.

Histology of muscle fibres. The *affected zone* at 2 days shows increasing separation, distortion, and break-up of many of the muscle fibres, a greatly increased polymorphonuclear response and much cellular debris. The muscle fibres fracture transversely or in the peculiar 'step' formation described by Le Gros Clark (1946) and Barer (1947). The intensely staining sarcomeres ('discs' of Bowman (1840), muscle 'clots' of Speidel (1937, 1939)) are particularly obvious in this zone of the muscle, but occasional fibres are found that appear to be very little altered in histological appearance. The intermediate and intact zones are unchanged from those seen at 18 hr.

Histology of nerve fibres. In the *affected zone* the condition of the nerves seen at 18 hr. remains largely unaltered. Empty neurilemmal tubes, sometimes containing fragments of debris, can be seen crossing areas of muscle break-up (Pl. 1, fig. 4). The sites of most of the end-plates cannot be identified. In the adjacent *intermediate zone* necrotic motor end-plates can be seen (Pl. 1, figs. 5, 6).

In the *intact zones* the innervation is similar to that in an intact muscle (Pl. 1, fig. 7). Occasionally empty neurilemmal tubes are seen, probably because the axons have had to traverse affected zones in their course.

In three animals 10 mm. of the extramuscular nerve was examined by the Weigert technique as described above, in order to determine how far proximally break-up had occurred. No difference was found between these nerves and intact controls from the opposite side of the body. Examination of the muscles in the region of the neurovascular hilum also showed little or no evidence of nerve break-up at this or at any later stage. It may therefore be concluded that degeneration does not proceed centripetally in the extramuscular part of the muscle nerve.

Six days after devascularization

Seven out of ten muscles responded to direct stimulation, and in four there was a faint contraction on indirect stimulation due to the integrity of some of the nerves supplying the intact zones. Considerable organization of perimuscular fibrous tissue has occurred, and many new dilated blood vessels pass into the damaged muscle from the surrounding fascia. The muscle surface after removal of this fibrous tissue remains similar in appearance to that seen on the second day. Bromophenol blue injected intravenously demonstrated, in all animals except one, that an

effective circulation has been established throughout the muscle, almost the whole of which stains an intense blue. One muscle showed a white muscle 'sequestrum', which remained uncoloured after injection of bromophenol blue intravenously (Pl. 1, fig. 8).

Histology of muscle fibres. There is an obvious difference between the great cellular activity now seen in the affected zone and the adjacent normal appearance of the intact zone. The muscle fibres passing from the intact zone taper and stream as narrow fibres into the affected zone (Pl. 2, fig. 9). The average diameter of these pale-staining fibres is about 15μ. Each contains a row of oval, centrally placed nuclei, arranged in groups of four or six. Transverse striations are prominent and are distorted to form a herring-bone pattern similar to that noted by Speidel (1939). These regenerating muscle fibres are at a stage analogous to the 'myotube' stage of myogenesis (Le Gros Clark, 1946). Interspersed among the young regenerating muscle fibres are large numbers of fibroblasts and capillaries.

At the centre of the affected area, masses of fragmenting and necrotic muscle fibres can be seen surrounded by leucocytes. These fibres are dark-staining (in contrast to the regenerating fibres) with no transverse striations or nuclei. Some show a fine longitudinally orientated granularity of the sarcoplasm. Multiple 'muscle clots', appearing as transverse discs of very dark staining material, occur at intervals in many fibres. The fibre diameters are highly irregular, and transverse fractures are common. The muscle with the 'sequestrum' showed in the affected zone masses of fibroblasts and leucocytes, with a few short lengths of regenerating muscle fibres.

Histology of nerve. Necrosis of nerve in the *affected zone* is usually complete and no axons are seen, though the outlines of groups of neurilemmal tubes can be made out against the highly cellular background of regenerating muscle fibres (Pl. 2, fig. 10).

In the *intermediate zones* empty neurilemmal tubes are seen in large numbers. But some contain axons which are probably regenerated following their initial break-up, and have grown down the short terminal length of neurilemmal tube to form motor end-plates.

In the *intact zones* the pattern of innervation is similar to that seen at 2 days.

Twelve days after devascularization

In seven out of twelve muscles there is a brisk contraction on indirect stimulation, and in eight muscles a localized contraction on direct stimulation of the muscle surface at scattered points. This response to indirect stimulation is due to the contraction of some fibres which have escaped necrosis and to others which have regenerated.

The perimuscular fibrous tissue is dense, but when this is dissected off, the muscle appears similar in colour to an intact muscle. As the fibrous tissue is removed there is a profuse bleeding from the raw surface.

Histology of muscle fibres. The reorganization in the *affected zones* is now quite advanced, but the pattern seen at 6 days has been maintained. Regenerating muscle fibres, masses of fibroblasts and some young fibrous tissue occupy much of the affected zones, but there are still visible large masses of necrotic vacuolated muscle fibres, the ends of which are surrounded by leucocytes (Pl. 2, fig. 11).

The regenerating muscle fibres are still narrow and contain many large oval nuclei, with twin dark-staining nucleoli, scattered through their substance. These nuclei are now often peripheral in position in contrast to the central position seen at 6 days.

Histology of nerve fibres. In the *affected zones* most of the nerve trunks can be seen only as the outlines of neurilemmal tubes, with necrotic Schwann nuclei present in large numbers, but regenerating nerve fibres can also be demonstrated amongst the actively growing muscle fibres and leucocytes. These axons are thin and varicose, and sometimes the outline of the myelin sheath and Schwann nuclei can be seen (Pl. 2, fig. 12). Occasionally a nerve fibre is seen to divide into a number of strands which come together again to reform the axon. The axons travel for considerable distances in the cellular tissue and end in a variety of ways, forks, bulbs and rings being observed (Pl. 2, fig. 13). In the *intermediate zones* the appearances first seen at 6 days of wide neurilemmal tubes with fine axons which are growing down to form new motor end-plates are now more common.

Twenty-one days after devascularization

At 21 days, after removal of the thickened epimysium, the muscles look normal in colour and size. Seven out of seven react to direct stimulation and six out of seven to indirect stimulation.

Histology of the muscle fibres. A considerable increase in reorganization was observed. In the *affected zones* there is a great excess of fibrous tissue surrounding the muscle fibres (Pl. 3, fig. 14) when compared with the intact zones. This fibrous tissue thickens the endomysium and the separation between fibres is thereby increased. In cross-section, the fibres are not facetted but round and of a more uniform size than normal. Most of the muscle nuclei are elongated and are arranged longitudinally at the periphery of the muscle fibre. Some nuclei, round or oval, are seen in the interior of the fibre.

Histology of the intramuscular nerves. Three definite patterns of nerves are seen. (1) In the *intact* and *intermediate zones* many of the original neurilemmal tubes have survived and they are packed with Schwann nuclei, but the arborization pattern appears normal. The axoplasm is dammed up, just before entering the end-plate, and then it forms a fine terminal claw, quite unlike the appearance seen in an intact muscle (Pl. 3, fig. 15). (2) In the *intermediate zones* neurilemmal tubes may conduct narrow varicose axons, which give rise to many motor end-plates (Pl. 3, fig. 16). Ultraterminal or 'escaped' branches (Gutmann & Young, 1944) are occasionally seen. (3) In the *affected zones* tangled skeins of varicose axons, often apparently without neurilemmal tubes, can be seen dividing and rejoining each other. Axons may terminate in motor end-plates where the claw of axoplasm has only two or three branches ending in a cluster of end-plate nuclei. But many axons do not seem to have an effective ending, and wander among the muscle fibres.

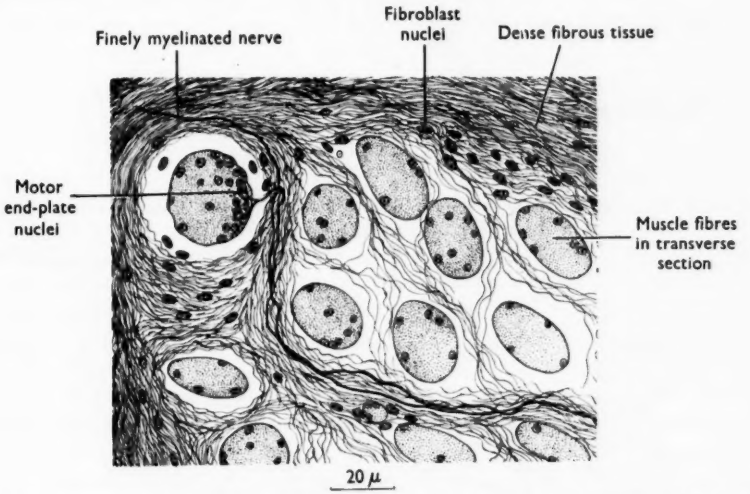
Highly suggestive histological evidence of the innervation of regenerating muscle fibres by regenerating nerve was found. In transverse sections of muscle at this time many fine axons were seen weaving their way through the thickened endomysial fibrous tissue surrounding the narrow-calibre muscle fibres, and some form small motor end-plates (Pl. 3, fig. 17 and Text-fig. 3). The claw is composed of two or

three short, fine strands of axoplasm, which end among about eight inner end-plate nuclei.

Thirty days after devascularization

At 30 days the naked-eye appearances are similar to those seen a week before. The muscles all respond to both direct and indirect stimulation.

Histology of muscle fibres. The intact and affected zones can still be distinguished. In the *affected zones* the increase in fibrous tissue and fibroblasts surrounding the muscle fibres is still obvious. On transverse section the muscle fibres are larger than at 21 days but still retain a rounded appearance and more or less uniform size. The number of oval muscle nuclei is apparently increased.



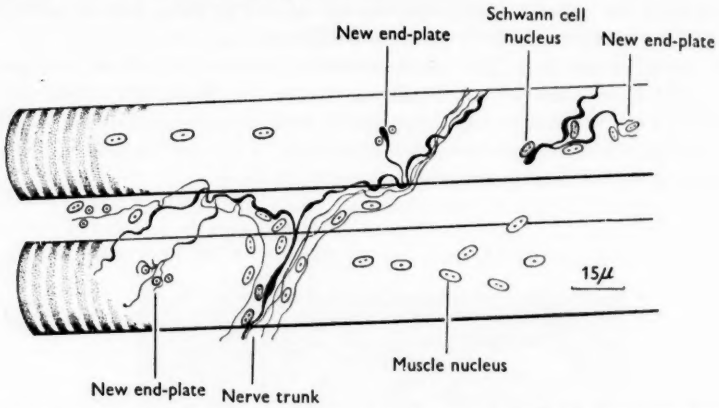
Text-fig. 3. Transverse section of muscle in affected zone 20 days after devascularization. Note the large amount of fibrous tissue, the small size of the muscle fibres and the muscle nuclei scattered throughout the fibres. Two nerves are seen crossing the field, one of which forms an end-plate after piercing the surrounding fibrous tissue. Compare with Pl. 3, fig. 17 (1665 E).

Histology of the nerves in the affected zones is characteristic. Occasional short stumps of narrow neurilemmal tubes, containing one or two axons, can be seen. There are considerable areas of muscle fibre innervated in the highly atypical manner already described. Axons, thinly myelinated, pass, mainly in a longitudinal direction, along the muscle fibres, and some form small solitary end-plates, often possessing an ultraterminal branch, whilst others end in a plexus (Pl. 3, fig. 18).

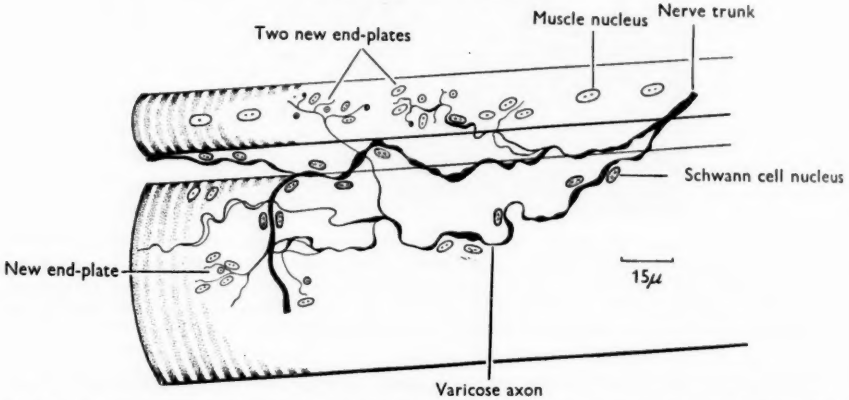
Thirty-eight and one hundred days after devascularization

The fibrous tissue surrounding the muscles is thickened, tough and white in colour, but strips off easily. Large blood vessels pass through the fibrous tissue into the muscles. These are somewhat reduced in size, but similar in colour and consistency when compared with an intact muscle. All muscles respond to direct and indirect stimulation with a brisk contraction.

Histology of the muscle fibres. After 38 days there is little more organization than at 30 days. A clear difference between intact and affected zones is, however, maintained even at 100 days after devascularization.



Text-fig. 4. Diagram showing a group of regenerated nerve fibres crossing the regenerated muscle. Simple end-plates are present.



Text-fig. 5. Diagram showing numerous regenerated end-plates on two regenerated muscle fibres. Note the fineness of the nerve which forms the end-plates and the terminal rings. Two motor end-plates end on one muscle fibre, and ultraterminal branching is present.

The *affected zones* are identified by the arrangement of muscle fibres. Usually only short segments of fibres are cut, probably because of the distortion produced by the contracture of the interstitial fibrous tissue (Pl. 3, fig. 19). The large size and oval shape of the nuclei of the fibres persists, and chains of nuclei are still seen at 100 days. Examination of transverse sections confirms the separation of muscle fibres from each other by fibrous tissue, though in longitudinal sections this is not so obvious in the 100-day muscles.

The *intact zones* show normal muscle, but in some cases this is separated off from the affected zones by a barrier of fibrous tissue.

Histology of the nerves. The appearances of the nerves seen 38 and 100 days after devascularization are comparable and are similar to those at 30 days. But in the *affected zone* groups of finely myelinated axons pass in a mainly longitudinal direction. They run in the fibrous tissue which separates the muscle fibres from one another, and they are most variable in size (Pl. 3, fig. 20). Some fibres possess obvious neurilemmal tubes with Schwann nuclei scattered along their length, but others are extremely fine and lie across and adjacent to muscle fibres. Aggregations of nuclei are present, and it is often very hard to tell whether a given group of nerve fibres is a motor end-plate or not (Text-fig. 4). Some axons have short branches, at right angles to the main trunk, which at once break up into a fine terminal end-plate. Other axons have been noted to end as a simple fine fibre, often with a small number of nuclei adjacent to it (Text-fig. 5). Large terminal plexuses similar to that seen at 30 days are occasionally seen.

CONCLUSIONS AND DISCUSSION

(1) *Initial effects of ischaemia*

Ligation of the main blood vessels at the neuro-vascular hilum of the tibialis anterior muscle of the rabbit produces varying amounts of damage, which are probably due to differences in the intramuscular vascular pattern. In the majority of animals, devascularization produced a central 'affected zone' containing a number of scattered lesions, having the appearance of a red infarct, with white necrotic and red haemorrhagic patches distributed over the surface of the muscle. In one animal a muscle 'sequestrum' was formed (cf. the human cases cited by Griffiths, 1940) which was greyish white in colour and had clearly defined edges, as late as the sixth day following operation. Barnes & Trueta (1942) used rabbits' hind-limbs in an experimental study of arterial spasm and found, on histological examination, large muscle 'sequestra' surrounded and infiltrated by fibrous tissue. They did not report muscle regeneration. Their observations agree with our finding that a muscle with a large 'sequestrum' showed little regenerative activity. It is probable that our case was due to localized vascular spasm preventing adequate revascularization.

In a few animals the amount of muscle affected by the devascularization was less than those described above. These are examples of muscles having highly efficient intramuscular vascular anastomoses.

We were able to confirm the previous work of Le Gros Clark & Blomfield (1945) which showed the astonishing rapidity with which these muscles are revascularized. Intravenous bromophenol-blue injection demonstrates that in many cases by the third day much of the muscle has an effective circulation. In others, such as that with the 'sequestrum' cited above, the process is much slower, but every muscle we examined after 10 days stained blue and was revascularized. Some were a more intense blue than even the intact muscles.

Cycles of muscle-fibre change

Two distinct cycles of muscle change probably occur in these experiments. (1) In the severely damaged areas (affected zones) cloudy swelling proceeds to necrosis and removal by phagocytes. Replacement is by regenerating muscle and

fibrous tissue. (2) In the less severely damaged areas (intermediate zones) cloudy swelling proceeds to recovery within a few days.

Extent of nerve necrosis

In the affected zones, as early as 18 hr. after devascularization, necrosis of the axons has occurred and the motor end-plates are difficult to locate. After 18 hr. there is no further involvement of intact axons, though the process of disintegration continues in those already affected. In the intermediate zones, the nerve fibres may be in a similar state to those of the affected zones. Frequently, however, only the terminal portions of the axons are necrotic and the motor end-plates are coarsely granular and appear enlarged. In the intact zones, the majority of fibres are unaffected, but an occasional necrotic axon may be seen.

Muscle response to electrical stimulation

Controlled indirect electrical stimulation at 2 days after devascularization demonstrated that sixteen out of twenty-two muscles did not contract (Table 1). The remaining muscles contracted probably because they had small affected zones, and therefore a high proportion of intact motor end-plates.

(2) *Regeneration of nerve*

Depending on whether the original neurilemmal tubes are available or not, different types of nerve reorganization are seen. In the *intact* and *intermediate zones*, the neurilemmal tubes remain though their arborization pattern may be distorted. (1) If only a short portion of the axon degenerates, the motor end-plate will be quickly reinnervated by the regenerating axon (Pl. 3, fig. 15). (2) When a greater length of axon degenerates, a number of fine axons grow down the old neurilemmal tube and groups of single claw endings are found (Pl. 3, fig. 16). The rapid regeneration of motor end-plates may be correlated with the increasing number of muscles which responded to indirect stimulation in the first 12 days (Table 1).

In the *affected zones*, where there is severe disorganization, some short lengths of neurilemmal tubes remain, but the typical arborization pattern is never seen. By the 12th day, finely myelinated axons can be seen crossing the cellular background. They all have simple tapering ends, without a complex end-plate (Pl. 2, fig. 13). At a later period, when the affected zone is becoming more organized, many fine axons can be observed weaving their way through the fibrous tissue (Pl. 3, fig. 17). They divide and reform to terminate either as simple motor end-plates with ultra-terminal branches, or as straggling lengths with irregular-shaped endings (Pl. 3, fig. 18). These end-plates have a curious atypical form. A number of nerve fibres end on groups of narrow muscle fibres, which are separated from each other by dense fibrous tissue. Other single endings are some distance from the nerve trunk (Text-fig. 3). It appears very probable that these are regenerated end-plates on new muscle fibres. Fragments of the old neurilemmal tubes occasionally transmit one or two fine axons, but usually they are blocked by fibrous tissue proliferation.

It would appear that in the affected zone nerve regeneration is similar in pattern to that seen in nerve implants into denervated muscle (Aitken, 1950). Nerve fibres grow into the affected zones, and many form motor end-plates with the new muscle

fibres, but in the later muscles examined (e.g. 38 days and later) it is difficult to be certain that a given muscle fibre has gone through the complete cycle of necrosis and regeneration. Histological appearances (diameter and nucleation of the muscle fibre, myelination and arrangement of axons in the nerve) are very suggestive.

In other cases end-plates do not form, but the axons pass across the muscle fibres and gradually taper away. Possible reasons for the latter behaviour may be:

(1) The axons are not motor fibres, but sensory or autonomic.

(2) The adjacent muscle fibre is already innervated in the intact zone and cannot be superinnervated.

(3) The dense fibrous tissue reaction impedes the growth of axons on to the muscle fibre.

This last possibility is of great interest.

(3) *Fibrous tissue proliferation*

Gutmann & Young (1944) showed the influence of the period of denervation on the efficiency of reinnervation and suggested that this was due to the fibrous tissue proliferation during disuse atrophy. In the cases under consideration there is a marked proliferation of fibrous tissue in a short period of time. Experiments to be reported in detail at a later date suggest that a muscle which has been denervated and devascularized rapidly undergoes severe atrophy and replacement by fibrous tissue. The atrophy can, in a large measure, be prevented by implantation of a new muscle nerve at operation.

It thus appears certain that the fibrous tissue proliferation found in mammalian muscle after injury is a major obstacle to the reorganization of a muscle during muscle and nerve-fibre regeneration.

From the data of Gutmann & Young (1944) it would appear that the tibialis anterior of a rabbit would be reinnervated in 16–18 days. In our experiments the nerve was not crushed, but the fibrosis which accompanies regeneration of muscle might retard the advance of the nerve fibres to a similar extent. Indirect stimulation shows that functional recovery had occurred 21 days after operation (Table 1).

Table 1. *Muscle responses to electrical stimulation*

Note that after the 21st day almost all the muscles respond to both direct and indirect stimulation.

Days after operation	No. of muscles examined	Direct stimulation		Indirect stimulation	
		No response	Contraction	No response	Contraction
2	22	8	14	16	6
6	10	3	7	6	4
12	12	4	8	5	7
21	7	0	7	1	6
30	6	0	6	0	6
38	6	0	6	0	6
100	4	0	4	0	4

The completeness of the recovery is probably determined by the extent of the fibrosis, which itself is dependent on the local vascular condition in the muscle. Examination of the muscles left for longer periods of time demonstrates the increased proliferation of fibrous tissue. The typical picture is of muscle fibres of small calibre growing through a mass of fibroblasts and fibrous tissue.

In larger muscles, such as the tibialis anterior of man, the extensive fibrosis resulting from arterial occlusion effectively prevents any extensive regeneration of the muscle fibres (Hughes, 1948), though it appears as if the regenerative process has in fact been initiated. The resulting histological picture is of a typical Volkmann's ischaemia of muscle. Experiments designed to control the deposition of fibrous tissue in damaged mammalian muscle might help to elucidate the factors involved in regeneration of muscle and its nerve supply.

SUMMARY

Following devascularization muscle goes through one of two cycles of change:

(*a*) Damage to nerve and muscle is slight and recovery follows without disorganization.

(1) When only the end-plate and adjacent nerve is damaged, the axoplasm appears to retract and regeneration quickly occurs with little alteration in the morphology of the end-plate. The axoplasm is often dammed up before entering the end-plate.

(2) Where the damage is more extensive and longer segments of axons are involved, regeneration results in a number of fine axons growing down the old neurilemmal tubes. Groups of single atypical end-plates are formed.

(*b*) Damage is extensive and necrosis is followed by regeneration of muscle and nerve.

(1) In the early stages the fine regenerating axons can be seen growing in the highly cellular tissue and have tapering endings between the regenerating muscle fibres.

(2) End-plates are not visible until about 21 days. They may be simple claw endings with numerous ultraterminal branches or rather complicated interlacings of a number of axons on the surface of the muscle fibres.

(3) After 28 days many of the end-plates are well organized with numerous terminal branches and end-plate nuclei. The pattern of innervation remains very atypical and it is doubtful whether further recovery occurs, owing to the development of fibrous tissue between the muscle fibres.

We wish to thank Prof. J. Z. Young for his help and encouragement in this work, and also Mrs P. Warren and Miss R. Robbins for technical assistance with the histological and photographic work.

REFERENCES

Aitken, J. T. (1950). Growth of nerve implants in voluntary muscle. *J. Anat., Lond.*, **84**, 38–49.

Aitken, J. T., Sharman, M. & Young, J. Z. (1947). Maturation of regenerating nerve fibres with various peripheral connexions. *J. Anat., Lond.*, **81**, 1–21.

Barer, R. (1947). Observations on muscle fibre structure. The swelling of muscle fibres by acids and alkalis. *J. Anat., Lond.*, **81**, 259–285.

Barnes, J. M. & Trueta, J. (1942). Arterial spasm, an experimental study. *Brit. J. Surg.* **30**, 74–79.

Bowden, R. E. M. & Gutmann, E. (1949). The fate of voluntary muscle after vascular injury in man. *J. Bone Jt Surg.* **31** B, 356–368.

Bowman, W. (1840). On the minute structure and movements of voluntary muscle. *Philos. Trans.* **130**, 457–501.

Clark, W. E. Le Gros (1946). An experimental study of the regeneration of mammalian striped muscle. *J. Anat., Lond.*, **80**, 24–36.

CLARK, W. E. LE GROS & BLOMFIELD, L. B. (1945). The efficiency of intramuscular anastomoses, with observations on the regeneration of devascularized muscle. *J. Anat., Lond.*, **79**, 15–33.

FISHBACK, D. K. & FISHBACK, H. R. (1932). Studies of experimental muscle degeneration. *Amer. J. Path.* **8**, 193–218.

GRIFFITHS, D. LL. (1940). Volkmann's ischaemic contracture. *Brit. J. Surg.* **28**, 239–260.

GUTMANN, E. & YOUNG, J. Z. (1944). The reinnervation of muscle after various periods of atrophy. *J. Anat., Lond.*, **78**, 17–43.

HARMAN, J. W. (1947). A histological study of skeletal muscle in acute ischaemia. *Amer. J. Path.* **23**, 551–565.

HUGHES, J. R. (1948). Ischaemic necrosis of the anterior tibial muscles due to fatigue. *J. Bone Jt Surg.* **30** B, 581–594.

KÜTTNER, H. & LANDOIS, F. (1913). Die Chirurgie der quergestreiften Muskulatur. 1. Teil. *Dtsch. Chir.*

ROUS, P. & GILDING, H. P. (1929). Studies on tissue maintenance. *J. exp. Med.* **50**, 189–211.

SPEIDEL, C. C. (1937). Studies of living muscles. I. *Amer. J. Anat.* **62**, 179–236.

SPEIDEL, C. C. (1939). Studies of living muscles. II. *Amer. J. Anat.* **65**, 471–529.

WALDEYER, W. (1865). Ueber die Veränderungen der quergestreiften Muskeln bei der Entzundung und dem Typhusprozess, sowie über die Regeneration derselben nach Substanzdefecten. *Virchows Arch.* **34**, 473–514.

YOUNG, J. Z. (1942). Functional repair of nervous tissue. *Physiol. Rev.* **22**, 318–374.

EXPLANATION OF PLATES

All sections are stained with a modified Bielschowsky-Gros technique, and the scale is shown on each figure.

Figs. 1–7. Conditions in muscle up to 2 days after devascularization.
Figs. 8–13. Conditions in muscle up to 12 days after devascularization.
Figs. 14–17. Conditions in muscle up to 24 days after devascularization.
Figs. 18–20. Conditions in muscle up to 100 days after devascularization.

PLATE 1

Fig. 1. Bromophenol-blue injection of leg 2 days after devascularization. Note the distinction between the affected zones (light) and the intact zones (dark). The transitional intermediate zone can also be seen (1904 B).

Fig. 2. Degenerating nerve trunk in the affected zone (18 hr.). The neurilemmal tubes are apparently intact but their contents are disintegrating (2365 B).

Fig. 3. Terminal necrosis of axons seen in the intermediate zone (18 hr.) (2365 B).

Fig. 4. Degenerating nerve trunk in affected zone (2 days). Note the two fine fibres which appear to have escaped (1754 A).

Fig. 5. Swollen end-plates with no axons visible; from the intermediate zone (2 days) (1819 A).

Fig. 6. As fig. 5.

Fig. 7. Motor end-plates in intact zone (2 days) (1751 B).

Fig. 8. Bromophenol-blue injection of leg 6 days after devascularization. Note the unstained muscle 'sequestrum' (1886 A).

PLATE 2

Fig. 9. In the affected zone (6 days). Note the longitudinally orientated regenerating muscle fibres in the lower part of the field and the darkly stained necrotic muscle fibres in the upper part. There is intense leucocytic activity (1749 A).

Fig. 10. Neurilemmal tubes with disintegrating axons in the midst of regenerating muscle fibres (6 days). Note the highly cellular background (1749 A).

Fig. 11. Mass of leucocytes surrounding end of degenerating muscle fibre (12 days) (2046 A).

Fig. 12. Regenerating nerves in the affected zone (12 days). Note the varicose axons, the bulbous endings and the numerous Schwann cell nuclei. Outlines of regenerated muscle are also seen (1792 B).

Fig. 13. Part of the affected zone (12 days). No regenerating muscle is visible, but long thin axons are seen crossing the highly cellular background (1791 C).

PLATE 3

Fig. 14. Transverse section of the affected zone (24 days). Note the excessive amount of fibrous tissue which surrounds the regenerated muscle (1652 D).

Fig. 15. Regenerated motor end-plates in the intact zone (21 days). Note the damming up of the axoplasm at the points of entering the end-plates (1913 H).

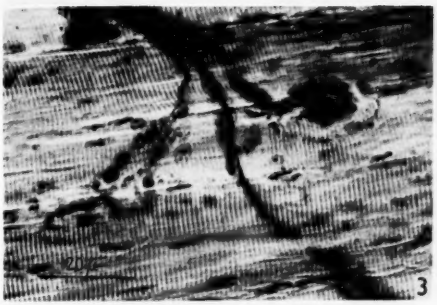
Fig. 16. Oblique section of the intermediate zone (20 days). Thin irregular varicose axons are seen forming numerous simple end-plates (1665 E).

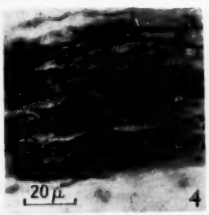
Fig. 17. Transverse section of the affected zone (20 days). Axons can be seen in the excessive fibrous tissue, and one winds its way through this fibrous tissue to form a simple end-plate. Compare with Text-fig. 3 (1665 E).

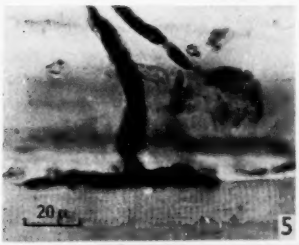
Fig. 18. Plexiform arrangement of axons in an end-plate (30 days) (2225 B).

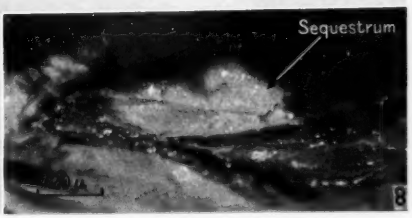
Fig. 19. The affected zone (100 days) shows organization of the excessive fibrous tissue surrounding and distorting the regenerating muscle and nerve fibres (2163 A).

Fig. 20. Regenerating muscle and nerve in the affected zone (100 days). The irregular diameter of the axons is probably due to the splitting and reuniting of individual axons (2163 A). Compare with Text-figs. 4, 5.









ALLBROOK AND AITKEN—REINNERVATION OF STRIATED MUSCLE AFTER ACUTE ISCHAEMIA

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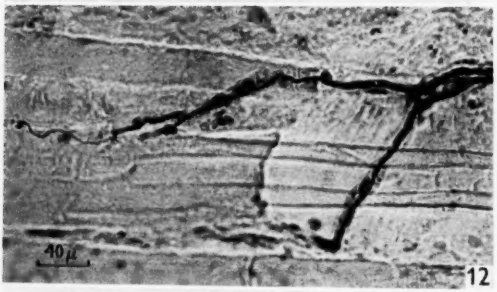

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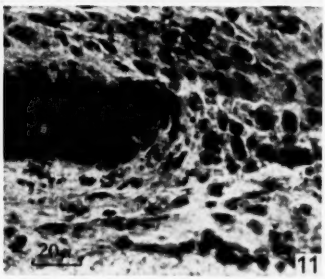

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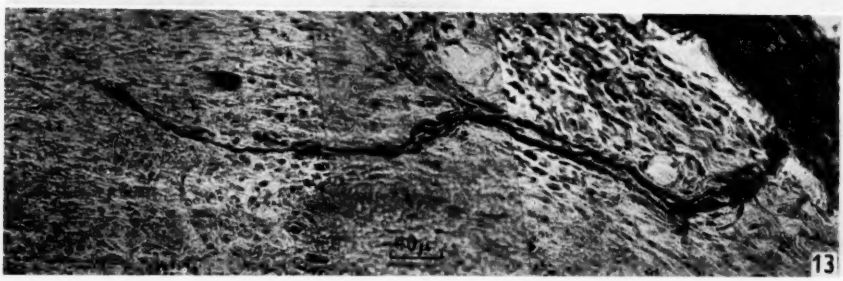

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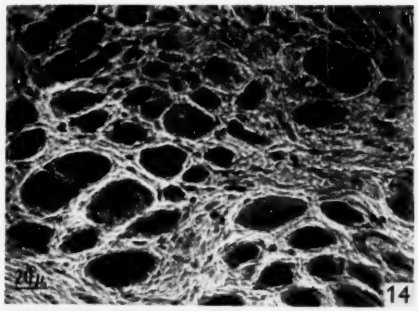
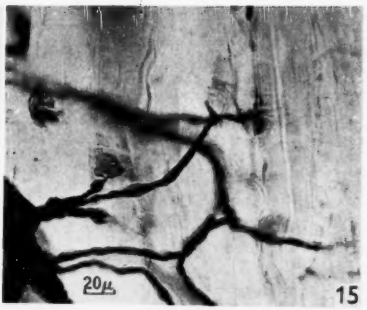
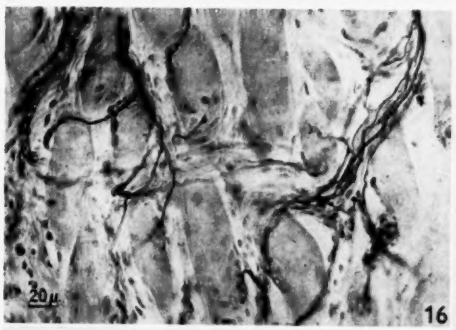
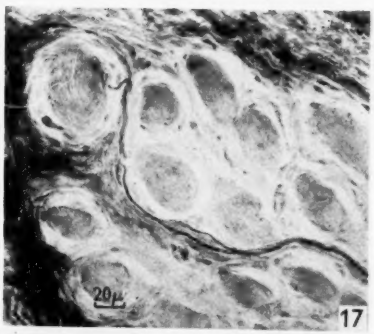
ALLBROOK AND AITKEN—REINNERVATION OF STRIATED MUSCLE AFTER ACUTE ISCHAEMIA









ALLBROOK AND AITKEN—REINNERVATION OF STRIATED MUSCLE AFTER ACUTE ISCHAEMIA

THE RELATIONSHIP OF BILE ALKALINE PHOSPHATASE TO HISTOCHEMICALLY DETECTABLE ALKALINE PHOSPHATASE IN THE BILIARY TRACT; INCLUDING REFERENCE TO THE HISTOLOGY OF THE GALL-BLADDER EPITHELIUM

By F. JACOBY and B. F. MARTIN
Department of Anatomy, University College, Cardiff

Prior to the era of the histochemistry of alkaline phosphatase (A.P.) it was known that the enzyme is excreted in the bile of certain species, e.g. man (Demuth, 1925) and dog (Umeno, 1931*b*; Armstrong & King, 1934).

The histochemical technique for A.P. applied to the liver has shown that the bile capillaries and, occasionally, the bile ducts can in some species be clearly demonstrated as the result of their A.P. activity. The most consistent positive reaction in this respect is found in the rabbit, as was first demonstrated by Gomori (1941); less consistent results have been reported for the liver of dog (Jacoby, 1946; Wachstein & Zak, 1946*a*) and man (Wachstein & Zak, 1946*b*; Sherlock & Walshe, 1947), though it seems that in these species a reaction is given more often than not, whilst guinea-pig liver stands out by the complete negativity of its bile capillaries and ducts (Bourne, 1943; Jacoby, 1946). Rat liver, too, is usually negative, though occasionally a few reacting bile capillaries can be found, especially at the periphery of a section (Jacoby, 1946).

It has been shown by a number of workers that histochemical data with respect to A.P. content in a tissue show some gross correspondence with the results obtained from chemical A.P. determination of the tissue extract. For the liver such chemical tests have given highest values in the case of the rabbit, and rather lower for cat, rat and man (Folley & Kay, 1936). Similarly, Umeno (1931*a*) graded the A.P. activity of liver tissues as follows: chick > rabbit > dog > guinea-pig > toad. These results fit in fairly well with the histochemical findings mentioned above; but chemical determinations on whole-liver tissue do not give a true indication of the A.P. content of the bile-capillary and bile-duct systems, since, apart from these, other structures (e.g. leucocytes, blood vessels and other connective tissue components) may, and do in fact, contain A.P. and so contribute to the overall chemical estimate of the organ's activity.

It was thought that perhaps a more precise relationship might exist between the actual A.P. content of bile and the histochemical picture of bile capillaries and bile ducts in the liver. The present investigation deals with a comparison of this kind, gall-bladder bile being used for the chemical determination of A.P. Because of the possibility of the gall-bladder epithelium contributing A.P. to bile, the gall bladder was also studied histochemically.

Three species were investigated: rabbit, dog and guinea-pig. The guinea-pig was of particular interest, first because of the lack of enzyme activity in the bile capillaries and bile ducts, and secondly because it has been reported that the gall-bladder

epithelium is A.P.-positive at its free surface and represents the actual source of the A.P. of the bile in this species (Bourne, 1943).

The likelihood of finding a positive reaction for A.P. at the distal border of the lining epithelial cells of the gall bladder brought to mind the still moot point regarding the presence or absence of a striated border in this epithelium. An additional histological examination of this epithelium was therefore made.

MATERIAL AND METHODS

Specimens of liver and gall bladder were obtained from twenty-one rabbits, nine dogs and twenty-four guinea-pigs, together with a sample of gall-bladder bile from each animal when possible. In the case of the guinea-pig it was sometimes necessary to pool the bile of several animals in order to have a quantity sufficient for the chemical test.

The tissues were fixed in 80 or 95% alcohol overnight, dehydrated, cleared in xylol and impregnated with paraffin wax for 2 hr. at 56° C. Sections were cut at a thickness of 7μ and mounted in series. The histochemical technique employed was that of Kabat & Furth (1941), sodium-β-glycerophosphate being the only substrate used. Sections from each tissue, together with controls, were incubated for $\frac{1}{2}$, 2 and 18 hr., and sometimes for periods in between these. One section of each series was stained with haematoxylin and chromotrop.

The chemical estimations of the A.P. content of dog and guinea-pig bile were done on centrifuged bile according to King's method (1947), a Klett colorimeter being used; and the results are expressed in King-Armstrong (K.A.) units per 100 cc. of bile. It was usually found necessary to dilute the dog's bile ($\times 10$) with saline prior to the estimation. In these cases a second determination was carried out with 0·04 cc. of undiluted bile instead of the usual 0·2 cc. There was quite good agreement between the results of the two determinations.

The A.P. estimations on the bile of the rabbits were done, with a similar method, by Mr J. W. Keyser of the Pathology Department of the Welsh National School of Medicine, to whom we are greatly indebted.

There does not yet exist a reliable method for estimating quantitatively the histochemically demonstrable A.P. in tissue sections, although some attempts have recently been made in this direction (Cleland, 1950; Gomori, 1950). For our purpose only a rough estimate was required, and this rests on visual appraisal. Because of the widespread and rather heavy reaction that usually occurred in the livers of rabbits and also in some of the dogs after incubation periods longer than 1 hr., evaluation of sections had to be made after $\frac{1}{2}$ hr. incubation. After that time, a definite grading of the extent of the bile capillary reaction in a lobule was possible, ranging from a few positive bile capillaries near the periportal connective tissue (marked as (+) in Table 1) to the complete mapping out of all bile capillaries in the lobule (marked as +++ in Table 1). This grading according to the extent of the bile-capillary reaction within a lobule seemed more reliable and certainly more feasible than a grading according to relative intensity (i.e. darkness) of the reaction.

The histological study of the gall-bladder epithelium was, in the first instance, made on those sections of the alcohol-fixed paraffin embedded material that were stained with haematoxylin and chromotrop. Both the fixative and stain turned out

to be very suitable for the purpose. With some dog material, however, other fixatives (alcoholic Bouin, formol, Helly's fluid, Zenker's fluid, and Flemming's solution with acetic acid) were also tried but—with the possible exception of alcoholic Bouin—did not prove superior to 80% alcohol.

Table 1

Rabbit no.	Bile A.P. (K.A. units /100 c.c.)	Histochemical A.P. reaction* in bile capillaries	Dog no.	Bile A.P. (K.A. units /100 c.c.)	Histochemical A.P. reaction* in bile capillaries
9	56	(+)	21	0	†
10	59	(+)	17	0	Pract.†
5	60	†	22	12	†
3	66	++	19	30	+
19	82	(+)	20	60	(+)
8	87	(+)	24	80	‡
7	90	(+)	18	110	+(+)
6	93	(+)	16	450	+++
24	107	(+)	23	900	++
17	119	+			
20	120	+(+)			
21	123	(+)			
23	127	(+)			
13	134	+(+)	Guinea-pig no.		
12	145	+	32–35	0·5	†
14	145	+	36	0·9	†
11	148	++(+)	37, 38	0·1	†
16	156	(+)	54	0·0	†
18	161	++	61	0·0	†
22	271	++	62	0·4	†
15	302	+++	63	0·0	†
	Mean 126·2				

The meaning of the + signs is explained in the text.

* Estimation after ½ hr. incubation.

† No reaction.

‡ Not investigated.

RESULTS

The results are represented in Table 1 and illustrated on Pls. 1 and 2.

(*a*) *Rabbit*

In twenty-one rabbits examined, the A.P. content of gall-bladder bile varied from 56 to 302 K.A. units/100 c.c. of bile, the average being 126·2 units.

It will be seen from Table 1 and Pl. 1, figs. 1–3, that there is a rough correspondence between the bile values and the extent of the bile-capillary reaction. This correspondence is, however, not absolute and perhaps cannot be expected to be so. The main discrepancies concern rabbits nos. 5, 3, 21, 23 and 16. No. 5 became definitely positive after 3 hr. incubation. The livers of rabbits nos. 21, 23 and 16 showed after 2 hr. incubation a positive bile-capillary reaction over a much wider area than after ½ hr., so that the discrepancies became less apparent. There are several factors which, singly or combined, might account for such discrepancies as were observed. Some are inherent in the technique, as, for instance, the degree of enzyme loss which has been shown to occur during the embedding process (Danielli, 1946; Stafford & Atkinson, 1948), and which, at present, is still an uncontrollable source of error and may vary from specimen to specimen. Consequently, the optimum incubation period is not always the same for different specimens. Other factors, possibly of

special importance, are physiological; in comparing gall-bladder bile with the histochemical bile-capillary picture, one has to realize that the A.P. content of gall-bladder bile is the resultant of an extended period of A.P. secretion by the liver and the storage and inspissation of the bile in the gall bladder; and that the A.P. secretion in the liver, responsible for the bile-capillary picture, may vary from time to time with different functional states. Thus, the liver of rabbit no. 3 may have been fixed at a time when there was a particularly strong A.P. secretion into the bile within the liver.

The outstanding feature, then, of Table 1 (rabbit) is the finding of invariably high bile A.P. values, and it seems that the consistency with which a bile-capillary picture can be obtained histochemically in this animal rests on this fact.

As far as the intrahepatic bile ducts are concerned, there is a fairly consistent A.P. reaction present at the free surface of the epithelium of the very small bile ducts (Pl. 1, figs, 1, 2) within the periportal connective tissue. The epithelium of the larger ducts was always found negative (Pl. 1, fig. 3).

Twenty-four gall bladders were examined. None of these showed a positive A.P. reaction in the epithelium. In some of them, a reaction, varying in extent, was present in the tunica propria (Pl. 2, fig. 7). In others, the entire gall-bladder wall was negative. It is worth noting that in some of the latter specimens a piece of liver tissue was included in the section, which gave the usual strong bile-capillary reaction, thus serving as a convenient control.

Histological note on the gall-bladder epithelium

The highly convoluted gall-bladder mucous membrane is lined by a simple columnar epithelium. The free surface of this epithelium appears, in side view, usually smooth, even and straight; only occasionally is the distal end of the epithelial cells somewhat rounded or dome-shaped which gives a scalloped appearance to the edge of the epithelial surface. In none of the specimens was there any evidence of a striated border.

(*b*) *Dog*

In contrast to the rabbit, dog's gall-bladder bile does not give consistently high A.P. values; in fact, the enzyme may be entirely absent (Table 1 shows a range from 0 to 900 K.A. units). Again, it will be seen that the extent of the histochemical bile-capillary reaction—as estimated after $\frac{1}{2}$ hr. incubation—roughly parallels the A.P. content of the bile (Table 1 and Pl. 1, figs. 4–6). Dog 22 is of interest. Here the bile capillaries of the liver remained negative even after long incubation periods. If, in this animal, the A.P. content of gall-bladder bile truly reflects that of the bile-capillary system—an assumption which can only be made with reservation—it would seem that the A.P. content of bile has to be higher than 12 K.A. units before a bile-capillary picture can be obtained.

The intrahepatic bile ducts of all sizes tend to give a positive A.P. reaction at the distal end of their lining epithelium (Pl. 1, figs. 5, 6).

In the gall bladder, the free border of the epithelium usually gives a patchy A.P. reaction (Pl. 2, fig. 8); this is sometimes more pronounced in the valleys of the mucosal folds than on the surface of their projections. These sites seem to be regions of genuine phosphatase activity and not due to ad- or absorption of enzyme from the bile, as will be discussed more fully below.

Histological note on the gall-bladder epithelium

The epithelium of the gall bladder is of the high columnar variety; its cells are often slender and closely set and sometimes show scalloping. Their average height is about 30μ. In all specimens there was to be seen a definite differentiation of the distal part of the epithelial cells into what can only be called a striated or brush border (Pl. 2, fig. 12). The height of this border was of the order of 2μ. The striations, though clearly visible in some places, were at others often very fine and faint, so fine that the border appeared almost as a homogeneous band; but it was always well demarcated by a fine, more deeply staining, line from the more granular cytoplasm below. The border was best seen when the cells were cut through a precisely vertical plane. If it seemed to fade out or to be absent, it was usually due to oblique sectioning of the epithelium. The border is, clearly, very similar to that of the intestinal epithelium; it is of the same height and also shows a basal demarcation line; the actual vertical striation, however, is much finer.

(*c*) *Guinea-pig*

The fact that, in this species, the bile capillaries and intrahepatic bile ducts are histochemically A.P. negative is now well known, and was confirmed in the present series (Pl. 2, fig. 9). The chemical tests for A.P. made on gall-bladder bile revealed that there is either no measurable amount present, or only a minute quantity (Table 1). It is not surprising that such minute traces of A.P. as may be present in bile are not detectable histochemically. These results seem to go a long way in explaining the A.P. negativity of the guinea-pig liver parenchyma.

Twenty-two gall bladders were examined, some from normal animals, and some from animals which had had the common bile duct ligated previously. These histochemical investigations involved the examination of a great number of sections, some of which had been incubated for as long as 3 days. With the exception of one specimen there was never a positive reaction given by the lining epithelial cells. In some cases the time of fixation and embedding was shortened, and also frozen sections were prepared and tested for A.P., with the same negative result. In most specimens, the only positively reacting material was represented by leucocytes in blood vessels (Pl. 2, fig. 10), and in a few instances by capillary endothelium and possibly reticular fibres in the tunica propria.

Only in a single gall bladder did we find a very small region with a distinct positive reaction at the surface of a small group of epithelial cells (Pl. 2, fig. 11). Incidentally, the bile of this gall bladder did not contain A.P. The specimen must be regarded as providing an exception to the general rule that the guinea-pig gall bladder shows no reaction for A.P. in the lining epithelial cells.

Histological note on the gall-bladder epithelium

The gall-bladder epithelium of this species appeared to be particularly plastic. Though columnar in empty and collapsed gall bladders, it was often found—at least in places—cubical or even flat with the long axis of the nuclei running parallel to the surface, if the organ was only slightly distended. The distal end of the epithelial cells showed a variety of appearances. Sometimes the edge was smooth and straight, and slightly more heavily stained than the rest of the cytoplasm; often

the distal end of the cells was dome-shaped, producing a scalloped edge of the epithelial surface; and, finally, bleb-like projections were often seen protruding from the distal cell surface and attached to it either by a broad base or by a narrow stalk, whilst similar blebs were seen completely detached nearby; the whole appearance being highly suggestive of a secretory process. The blebs, however, did not stain with mucicarmine.

In no instance could a striated border be discerned.

DISCUSSION

These investigations show that the species examined differ greatly in the A.P. content of gall-bladder bile. None, or only minute amounts, is found in that of the guinea-pig. This species seems to have been little investigated from this point of view. The only data we were able to find in the literature are given in a paper by Navarro (1946). His figures, expressed in Bodansky units, show an average value of 8·6, which is considerably higher than is recorded here. A definite explanation of this discrepancy cannot be given, but it should be said that our determinations were always done on bile after centrifugation. If this is not done, higher values may be obtained owing to possible contamination with leucocytes or with enzyme liberated from their breakdown. Navarro does not state whether he used centrifuged bile.

In striking contrast to that of the guinea-pig, rabbit bile always contains considerable amounts of the enzyme. It is this that makes the liver of this species so suitable—in fact, the one of choice—for demonstrating bile capillaries by means of the histochemical reaction for A.P.

The dog is intermediate between rabbit and guinea-pig in that its bile values may vary from 0 to 900 K.A. units or more. Until recently, the figures reported for bile phosphatase values in dogs have given the impression of being consistently fairly high (Armstrong & King, 1934; Freeman & Chen, 1938; Cantarow & Miller, 1948), ranging approximately from 50 to 300 K.A. units; but Dalgaard (1949), investigating the bile of normal dogs, points out the enormous variations that may occur, which, in his series, usually ranged between 0 and 185 Buch units; but in a few estimations reached even higher values, up to 1700 units. With these latter results our own findings are in good agreement, and the more irregular histochemical pictures which are obtained with dog's liver are thus readily explained.

The phosphatase reaction given by the epithelium of bile ducts and gall bladder in the three types examined does not parallel that of the corresponding liver (i.e. bile capillaries). Here the dog shows the most extensive reaction; the intrahepatic bile ducts of all sizes being fairly consistently positive at the distal surface of their epithelium, and also the gall bladder showing a positive though patchy reaction at the border of the lining epithelial cells.

In the rabbit, only the very small intrahepatic bile ducts are positive; the epithelium of the larger ones and of the gall bladder is negative.

In the guinea-pig neither bile duct nor gall-bladder epithelium gives a reaction. Reference has already been made to the single instance of a positive finding in a small patch of epithelial cells of one gall bladder. This we are inclined to interpret

as a developmental freak, having in mind the entodermal fore-gut origin of this epithelium. Our results, therefore, provide no evidence, so far as the guinea-pig is concerned, for the theory that the epithelium of this part of its biliary tract is a source of A.P. For the other two species, a possible secretion of A.P. from the bile ducts cannot be excluded, though no definite proof exists. The difficulty of interpreting such findings will become apparent by discussing the positive A.P. reaction seen in the epithelium of the dog gall bladder. It is unlikely that it represents merely an adsorption of the enzyme on to the epithelium from the bile contents, since such positivity was encountered in cases where there was no A.P. in the bile. Also, the fact that the lining epithelium of the rabbit gall bladder is consistently negative, in spite of the regularly high A.P. content of the bile, lends indirect support to this view. The finding, in some of the dogs, of a positive gall-bladder epithelium, but A.P.-negative bile, might be used as an argument against the idea that the epithelium contributes phosphatase to the bile by means of secretion. There remains another possibility, namely, that the A.P. activity histochemically displayed by the distal border of the dog gall-bladder epithelium is concerned with some selective process of absorption. Though the functions commonly assigned to the gall bladder are those of storage and inspissation of bile, there exist observations relating to its capacity for absorbing material other than water through its epithelium. As early as 1857, Virchow, in his classical description of the structure of the gall-bladder epithelium, made reference to the appearance of fat granules and droplets in the epithelium, the picture strikingly resembling that of fat absorption in the intestinal epithelial cell. Pfuhl (1932), in his review of the gall bladder, quotes various observations on fat resorption by gall-bladder epithelium which physiologically occurs after fatty meals in carnivores (not normally in the rabbit), the fatty substances having been transported to the gall bladder from the liver via the bile. Experimentally, the fat absorption was also seen after the introduction of milk into the gall bladder, and Pfuhl states that in this process the epithelium is not involved uniformly, but in a rather patchy manner. Having examined frozen sections of dog's gall bladder stained with scarlet R, we can confirm the observation that a considerable accumulation of fat droplets and granules can be demonstrated in this epithelium.

It seems of interest to mention these observations in view of our findings with respect to the histochemical distribution of A.P. in the dog's gall bladder. It is quite possible that the enzyme found here may play an important role in the transfer of fatty substances, some of which are phosphate-containing lipids, from the bile into the cytoplasm of the epithelial cells.

This functional aspect of the absorbing capacity of the gall-bladder epithelium leads now to a discussion of the structural problem attached to it, namely, that concerning the presence or absence of a striated border. The accounts given in text-books of histology vary, some describing such a border while others do not, and some even deny its existence. The negative view has recently been challenged by Ferner (1949), who reported for the human gall bladder the presence of a cuticular border, and by Ralph (1950), who studied the gall-bladder epithelium of one human and two monkey specimens. Ralph found that although no differentiation at the distal border of the cells could be seen with the bright field microscope, the use of

phase contrast revealed an even, narrow fringe of surface filaments which were from 0·4 to 0·8 μ long in the monkey, and from 0·5 to 1·5 μ in man. The filaments did not seem to be fused together, nor embedded in any matrix. When the epithelium lining the ileum of the monkey was examined by phase contrast, the 'striated border' was seen to be morphologically similar to that of the gall bladder, though the filaments were longer and more closely set. Examination by phase contrast of the lining epithelium of the pylorus (an additional control study) did not reveal the presence of surface filaments.

Our present observations were made without the use of phase contrast. By means of the bright field microscope we were able to distinguish a clearly represented striated border in the dog's gall-bladder epithelium, much higher (i.e. 2 μ+) than that seen by Ralph in the monkey or in man, whereas no such special differentiation was seen in the rabbit or guinea-pig.

It seems clear, then, that there exist species differences with regard to the surface structure of the gall-bladder epithelium, which at least in part account for the divergent views expressed about it.

Only further work with phase contrast or electron microscopy can provide us with more information in those cases which do not show a striated border with the bright field microscope.

It is worth recalling that Virchow (1857), in what is probably the oldest detailed description of this epithelium, based on a study of man, dog and cat, stated that it resembles structurally the intestinal epithelium, and that this can be particularly well seen in the dog.

It seems likely that in the dog's gall bladder the presence of a striated border, A.P. activity at the border, and fat accumulation in the epithelium are all related to one another.

A final point which arises from the foregoing comparative study concerns its pathological as well as physiological application. It will be obvious that the guinea-pig, having histochemically an A.P.-negative biliary tract and little or no A.P. in its bile, should be an interesting species for an experimental study pertaining to the still unsolved problem of the rise in serum A.P. which occurs in man and some experimental animals when the common bile duct is obstructed. The results of experiments of this kind, made on the guinea-pig, will be the subject of a separate communication.

SUMMARY

1. The relationship of the alkaline phosphatase content of gall-bladder bile to the histochemical A.P. picture given by bile capillaries, intrahepatic bile ducts, and gall-bladder epithelium has been studied in three species: rabbit, dog and guinea-pig.

2. The enzyme was found to be always present in large amounts in rabbit's bile; very variable in amount (including zero) in that of the dog; and absent, or present only in traces, in that of the guinea-pig.

3. The bile A.P. content could be roughly correlated with the histochemical bile-capillary picture in the corresponding liver, but not with the A.P. reaction of the corresponding gall-bladder epithelium.

4. A positive A.P. reaction of the gall-bladder epithelium was only found in the dog.

5. The gall-bladder epithelium of the three species was also investigated histologically, and it was found that only in the dog does it show a striated border.

6. The findings are discussed and related to physiological aspects.

We wish to express our thanks to Mr L. Jones for his help with the histological preparations, and to Mr A. Welch for the microphotography.

REFERENCES

ARMSTRONG, A. R. & KING, E. J. (1934). Phosphatase in obstructive jaundice. *Canad. med. Ass. J.* **31**, 14–20.

BOURNE, G. (1943). The distribution of alkaline phosphatase in various tissues. *Quart. J. exp. Physiol.* **32**, 1–20.

CANTAROW, A. & MILLER, L. L. (1948). Non-excretion of jaundice-serum alkaline phosphatase in bile of normal dogs. *Amer. J. Physiol.* **153**, 444–446.

CLELAND, K. W. (1950). A study of the alkaline phosphatase reaction in tissue sections. Part I. The possibility of its quantitative use. *Proc. Linn. Soc., N.S.W.* **75**, 35–53.

DALGAARD, J. B. (1949). Serum and bile phosphatase in biliary fistula dogs. *Acta physiol. scand.* **16**, 293–307.

DANIELLI, J. F. (1946). A critical study of techniques for determining the cytological position of alkaline phosphatase. *J. exp. Biol.* **22**, 110–117.

DEMUTH, F. (1925). Ueber Hexosephosphatasen in menschlichen Organen und Körperflüssigkeiten. *Biochem. Z.* **159**, 415–423.

FERNER, H. (1949). Ueber das Epithel der menschlichen Gallenblase. *Z. Zellforsch.* **34**, 503–513.

FOLLEY, S. S. & KAY, H. D. (1936). The phosphatases. *Ergebn. Enzymforsch.* **5**, 159–212.

FREEMAN, S. & CHEN, Y. P. (1938). The effect of jaundiced blood upon normal dogs with special reference to the serum phosphatase. *J. biol. Chem.* **123**, 239–246.

GOMORI, G. (1941). The distribution of phosphatase in normal organs and tissues. *J. cell. comp. Physiol.* **17**, 71–83.

GOMORI, G. (1950). The quantitative determination of histochemical reactions for enzymes. *Exp. Cell. Res.* **1**, 33–36.

JACOBY, F. (1946). Differences in localization, by histochemical means, of alkaline phosphatase within the same organ of different experimental animals. *J. Physiol.* **105**, 19*P*.

KABAT, E. A. & FURTH, J. (1941). A histochemical study of the distribution of alkaline phosphatase in various normal and neo-plastic tissues. *Amer. J. Path.* **17**, 303–318.

KING, E. J. (1947). *Micro-analysis in Medical Biochemistry.* London: J. and A. Churchill Ltd.

NAVARRO, J. L. (1946). La secreción intestinal de fosfatases. *Rev. Esp. Fisiol.* **2**, 211–282.

PFUHL, W. (1932). Die Gallenblase und die extrahepatischen Gallengänge. In v. Möllendorff's *Handbuch der mikroskopischen Anatomie des Menschen.* **2**, 426–462. Berlin: J. Springer.

RALPH, P. H. (1950). The surface structure of the gall bladder and intestinal epithelium of man and monkey. *Anat. Rec.* **108**, 217–226.

SHERLOCK, S. & WALSHE, V. (1947). Hepatic alkaline phosphatase: Histological and microchemical studies on liver tissue in normal subjects and in liver and in bone disease. *J. Path. Bact.* **59**, 615–630.

STAFFORD, R. D. & ATKINSON, W. B. (1948). Effect of acetone and alcohol fixation and paraffin embedding on activity of acid and alkaline phosphatases in rat tissues. *Science*, **107**, 279–281.

UMENO, M. (1931*a*). Ueber die Leberphosphatase der verschiedenen Laboratoriumstiere. *Biochem. Z.* **231**, 324–327.

UMENO, M. (1931*b*). Ueber das Vorkommen der Phosphatase in der Galle und im Pancreassaft. *Biochem. Z.* **231**, 346–351.

VIRCHOW, R. (1857). Ueber das Epithel der Gallenblase und über einen intermediären Stoffwechsel des Fettes. *Virchows Arch.* **11**, 574–578.

WACHSTEIN, M. & ZAK, F. G. (1946*a*). Histochemical distribution of alkaline phosphatase in dog liver after experimental biliary obstruction. *Proc. Soc. exp. Biol., N.Y.*, **62**, 73–76.

WACHSTEIN, M. & ZAK, F. G. (1946*b*). Distribution of alkaline phosphatase in the human liver. A study of postmortem material. *Arch. Path. Lab. Med.* **42**, 510–516.

EXPLANATION OF PLATES

All figures, except fig. 12, are of sections treated to show the presence of alkaline phosphatase (A.P.). No counterstain was used. The photographs 1–11 were taken through a Wratten G filter, and 12 through a Wratten B.

Plate 1

Fig. 1. Rabbit no. 9, liver. ½ hr. incubation. A bile-capillary reaction is seen only at the periphery of the lobule. Two small bile ducts show a reaction at the inner surface. × 125. Bile A.P., 56 K.A. units.

Fig. 2. Rabbit no. 20, liver. ½ hr. incubation. The bile-capillary reaction extends over the peripheral half of the lobule. Smaller bile ducts show a reaction. × 125. Bile A.P., 120 K.A. units.

Fig. 3. Rabbit no. 15, liver. ½ hr. incubation. Extensive bile-capillary reaction throughout the lobule. Part of a larger bile duct with negative epithelium can be seen. × 125. Bile A.P., 320 K.A. units.

Fig. 4. Dog no. 21, liver. ½ hr. incubation. No bile capillary reaction. × 125. Bile A.P., 0 K.A. units.

Fig. 5. Dog no. 19, liver. ½ hr. incubation. A patchy bile capillary reaction, largely confined to the periphery of the lobule. A bile duct shows a reaction in the epithelium. × 125. Bile A.P., 30 K.A. units.

Fig. 6. Dog no. 16, liver. ½ hr. incubation. Bile-capillary reaction extends throughout the lobule. Two medium-sized bile ducts show a reaction at the surface of the lining epithelium. × 125. Bile A.P., 450 K.A. units.

Plate 2

Fig. 7. Rabbit no. 13, gall bladder. 2 hr. incubation. No reaction given by the lining epithelial cells. In this specimen a corium reaction was obtained. × 80.

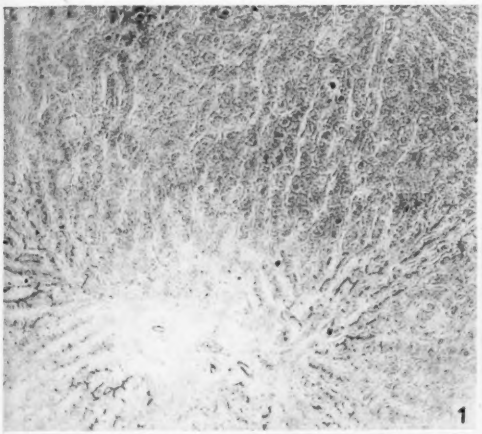
Fig. 8. Dog no. 18, gall bladder. ½ hr. incubation. Patchy reaction at the distal border of the lining epithelial cells, together with patchy corium reaction. × 80.

Fig. 9. Guinea-pig, liver. 24 hr. incubation. No reaction in either bile capillaries or bile ducts. The only reaction present is given by polymorphonuclear leucocytes. × 190.

Fig. 10. Guinea-pig no. 13, gall bladder. 20 hr. incubation. No reaction given by lining epithelial cells or by corium tissue. Again, only leucocytes in veins are positive. × 80.

Fig. 11. Guinea-pig no. 63, gall bladder. 22 hr. incubation. In this exceptional case, a very small region is seen (▭) where the lining epithelial cells show a reaction at the distal border. × 15.

Fig. 12. Dog no. 34, gall bladder. Fixation 80% alcohol; haematoxylin, chromotrop. The epithelium shows a definite striated border. The fine and coarse vacuoles in the more granular cytoplasm underneath the border and above and below the nuclei are due to dissolved out fat droplets. × 1250.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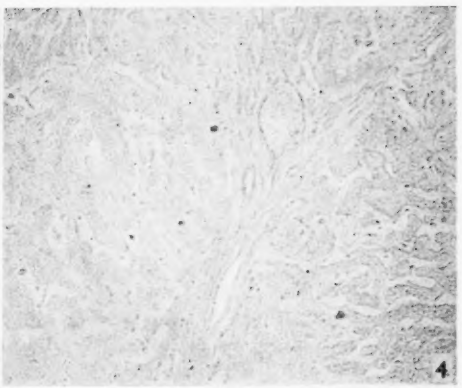
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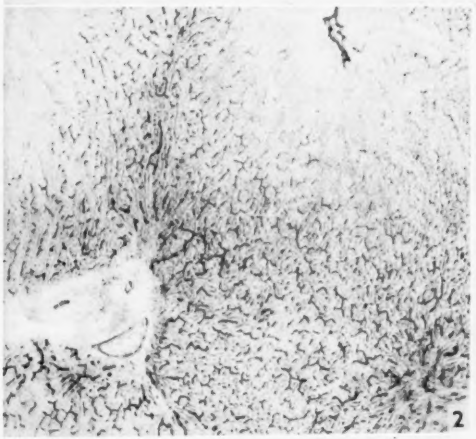
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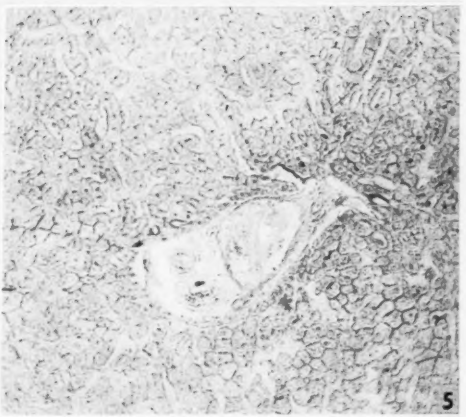
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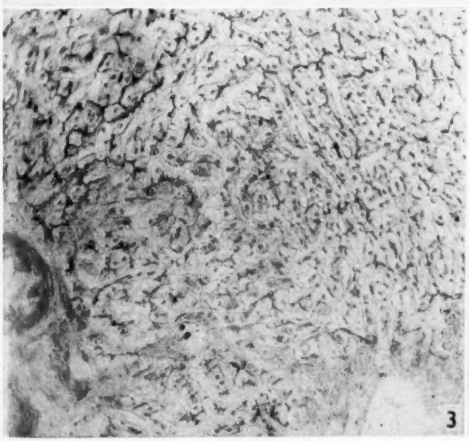
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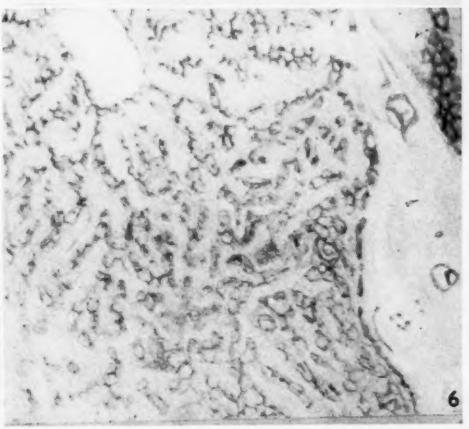
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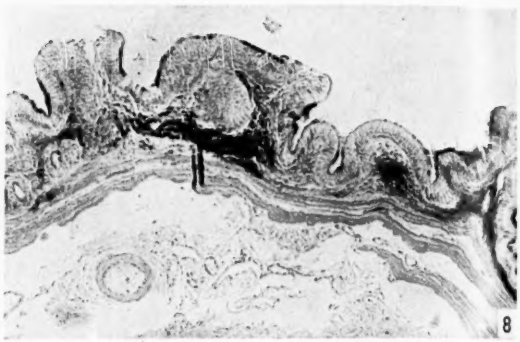
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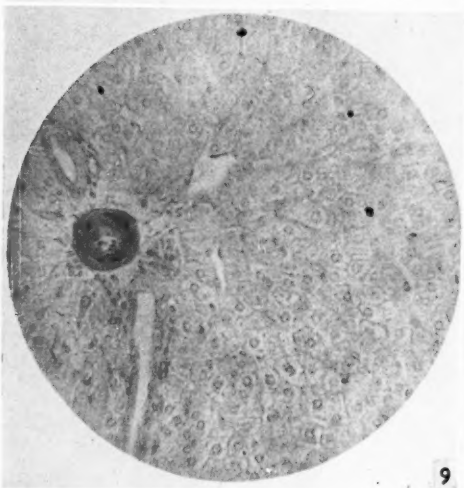
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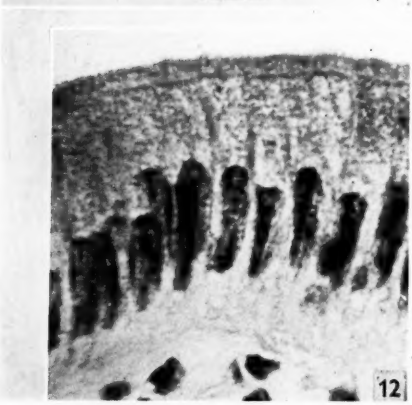
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Some time ago the present writer had occasion to set down his sense of privilege in having been a Dublin student at the start of the century. His note, however faultily expressed, was so conditioned by four years of work with Evatt that this may be a fit place to record it: 'What students ever had more widespread opportunity of contact with men and teachers of such fine integrity and sheer disinterested loyalty of heart?... When we were young we took those men too easily for granted. Later we came to recognize them as the rare exemplars of a presence which lit their infinite variety, a spirit ominously fading with the lamps of Europe—the academic spirit."

That of course is only a facet of Evatt's influence. He who was so kindly, so understanding, and so quietly inspiring had much in common with the person whose presence is evoked by Kipling's 'small hard man with eyebrows'. This meeting ground, which holds perhaps the secret of his character, is, as the Scots would say, outwith the mundane scope of paragraphs like these: it is another story.

A.K.H.

The above Note appeared in the *Irish Journal of Medical Science* and we are indebted to the Editor for his kind permission to reproduce it. Photograph by Lafayette, Dublin.

REVIEWS

Fossil Mammals of Africa. No. 1. *The Miocene Hominoidea of East Africa.* By W. E. Le Gros Clark and L. S. B. Leakey. (Pp. v+117; 28 text-figures, and 9 Plates; $12\frac{1}{4}+9\frac{1}{4}$ in.; 25*s*.) London: British Museum (Natural History). 1951.

This well produced monograph will be welcomed by all those interested in the evolution of the higher Primates. It brings together in one volume information which has already appeared in various publications and that dealing with the most recent finds made during 1947 and 1948 by the members of the British-Kenya Miocene Expedition.

It is a clear, well written and well illustrated account of what now amounts to a large quantity of material, albeit mainly teeth and jaw fragments, found at Rusinga Island and other localities in Kenya.

The specimens described comprise representatives of the genera *Proconsul*, *Sivapithecus* and *Limnopithecus*.

As the authors pointed out in a previous publication (1950) some rearrangement of genera and species has been made since MacInnes's description (1943) of *Proconsul africanus*. Thus, the specimens of *P. africanus* of which Dr Leakey had made casts available, and which comprised a damaged maxillary portion of a skull and a more complete mandible, now constitute a new species *P. nyanzae*; Hopwood's genus *Xenopithecus* has disappeared and is included with *Proconsul*. A new species of *Limnopithecus* does honour to the labours of MacInnes as *L. macinnesi*. One specimen illustrated by MacInnes (1943) as belonging to *Proconsul africanus* is now regarded as a new species, *africanus*, of the genus *Sivapithecus*. We thus now have the following Miocene representatives of the Hominoidea from East Africa:

Proconsul africanus Hopwood
P. nyanzae Le Gros Clark & Leakey
P. major Le Gros Clark & Leakey
Sivapithecus africanus Le Gros Clark & Leakey
Limnopithecus legetet Hopwood (including *L. evansi* MacInnes)
L. macinnesi Le Gros Clark & Leakey

An important addition to the specimens of *Proconsul africanus* already described is the skull, found by Mrs Leakey in October 1948, of which several good photographs are shown. Newly included as *P. africanus* is a mandibular fragment allocated by MacInnes to *Xenopithecus*. Other fragments, one an almost complete mandible, except for the ascending rami, from Rusinga Island, and a number of isolated teeth complete the specimens of *Proconsul africanus*.

P. nyanzae comprises the maxillae and mandible illustrated, and described by MacInnes (1943) under the name of *P. africanus*, and eleven other fragments and isolated teeth.

The representatives of *P. major* were all newly discovered in 1947 and comprise three mandibular fragments and some isolated teeth.

Sivapithecus africanus is represented by a maxillary fragment carrying the left premolar and 1st molar teeth in an excellently preserved condition, and two isolated right upper molars, one very well preserved.

Limnopithecus legetet is represented by eleven mandibular fragments and some isolated teeth. Three of the specimens were described and illustrated by MacInnes (1943); the remainder are new discoveries and their identity is not in all cases certain.

L. macinnesi comprises six mandibular and three maxillary fragments, and several isolated teeth. Some of the specimens are particularly well preserved.

In addition some tarsal bones, a right talus and calcaneum from the same body, and a left talus described by MacInnes (1943), and parts of two femora, a humerus and a clavicle discovered in 1933 by Archdeacon Owen, have been provisionally allocated to the genus *Proconsul*.

This whole collection forms an imposing array, and the authors of this monograph are fortunate in being able to study the actual specimens. For most of us there are the casts only of what now constitutes *P. nyanzae*, and one cannot but recognize how much easier and more convincing it is to follow the authors' account and argument when one can examine even a cast and compare it with actual chimpanzee and gorilla material instead of comparing photographs, which can never bring out the cusp patterns like the specimens themselves. Some of the photographs do show the cusp pattern as well as any photograph could, particularly those of *P. major* and *Sivapithecus africanus*.

The monograph begins with a brief account of the circumstances of the discoveries, the age of the deposits, the distribution of the finds among the various localities and a plan of Rusinga Island showing the wide distribution of sites upon it.

Then follows the diagnosis of the various types, which is almost a reprint of the authors' previous publication (1950).

The main body of the paper (85 pp.) gives a detailed description of the specimens, with some remarks on their relationships, and the monograph concludes with 15 pages devoted to an interesting discussion on the relation of the Miocene Hominoidea of East Africa to the evolution of the Primates.

Some account of the authors' several conclusions may now be given. It seems that all this material provides us with evidence that at this early date there was already a profusion of hominoid types. In *Limnopithecus legetet* the dimensions of the teeth approach the lowest limit of variation in the smaller species of modern gibbon, while the lower molars of *Proconsul major* approach in size those of the gorilla.

Broadly speaking, one may construct an approximate morphological sequence from *Parapithecus*, through *Propliopithecus*, *Limnopithecus legetet*, and *Pliopithecus* to *Hylobates*, but it is not of course suggested that this represents an actual linear evolutionary series. It does, however, seem certain that, at least on the basis of dental structure, *Limnopithecus* is quite closely related to *Propliopithecus* and *Pliopithecus* and may be provisionally included in the subfamily Hylobatinae.

It appears that the major portion of the Miocene anthropoid ape population was composed of members of the genus *Proconsul*, which varied from *africanus* of about gibbon-like size to the gorilla-like *major*. All these forms were approximately contemporaneous.

The specific distinctions of *Proconsul* rest mainly on cusp pattern rather than tooth size, for there is such a gradual transition in the latter as to make it difficult to determine the limits between one species and another. The main distinctive feature of the genus is to be found in the strong development of the internal cingulum in the maxillary molars.

The general resemblance between the mandibular teeth of *Proconsul* and those of *Dryopithecus* and *Sivapithecus* is very striking, but in several features they show significant differences. Perhaps the most conspicuous of these is the small size of the first molar in *Proconsul*.

While comparison of the limb bones of *Proconsul* and *Dryopithecus* must, on account of the paucity and uncertainty of the material, be very tentative, there does appear to be a remarkable similarity in size and proportions between the humerus and femur of *Proconsul* and of *Dryopithecus*.

With regard to the origin of *Proconsul*, it was presumably derived from some advanced hylobatine type similar to *Limnopithecus*.

It may be suggested that *Propliopithecus* gave rise to a more advanced hylobatine ape of the *Limnopithecus* type which provided the basis for the evolutionary development of *Proconsul*, and that the species of *Limnopithecus* which existed contemporaneously with *Proconsul* represent survivals, not greatly modified, of this ancestral stock.

With regard to the relationship of *Proconsul* to the modern representatives of the Hominoidea, *Proconsul* agrees more closely with the gorilla than the chimpanzee. In its general features there appears no sound reason against the suggestion that the *Proconsul* group provided the ancestral stock from which the modern African anthropoid apes were derived, though account must be taken of the apparent specialization in the molar teeth of *Proconsul*. If *Proconsul* may be accepted as a representative of the evolutionary stage

reached by the Hominoidea in early Miocene times, this is clearly pertinent to the problem of the evolutionary origin of the whole group of anthropoid apes and also of man himself.

From a study of the skull of *Proconsul* and of the jaws it seems reasonable to infer that the characteristic features of the skulls of the African apes of to-day are all secondary features which appeared at a later stage of evolution.

It seems probable that *Sivapithecus africanus* had a common origin with *Proconsul* and that it also provided the ancestral stock for which the European and Asiatic Dryopithecinae were derived. It certainly had very close affinities with *Sivapithecus indicus*.

The limbs of the early Miocene apes appear very different from those of the recent Pongidae, the straightness and slenderness of the shafts of the humerus and femur indicate relatively lightly built and agile creatures. Other features of the femur and of the talus and calcaneum conform with a cercopithecoid type of limb skeleton associated with quadrupedal locomotion, and at the same time provide no evidence that a brachiating mode of life had been adopted. The early Miocene apes of East Africa appear to have been active, running and leaping creatures in no way specialized for a purely arboreal habitat.

The Hominoidae diverged from the Pongidae perhaps in the Miocene, when the early anthropoid apes still possessed a limb structure similar to that of *Proconsul*. From such a group the modern anthropoid apes developed with their characteristic brachiating limbs, while in another direction the precursors of the Hominoidae arose, without passing through a brachiating phase but by the gradual conversion of limbs of the *Proconsul* type into limbs adapted for upright progression.

C. M. WEST

We are asked to draw our readers' attention to the recent issue of an English translation by Stewart Gilder of Professor Lacroix's *L'Organisation des Os* of which a review appeared in Volume 85, Part 1. This translation is published by Messrs J. & A. Churchill at 25*s*. net.

The Neural Crest. By SVEN HÖRSTADIUS. (Pp. x+111; 46 figures; 8vo; 15*s*. net.) Oxford University Press. 1950.

Those of us who had the pleasure of hearing Prof. Hörstadius's series of Special University Lectures at the University of London in 1947 will welcome their reappearance in a more permanent form. As the author states in his preface, the neural crest receives little more than passing mention in many text-books of embryology or anatomy, so that the wide and detailed survey given in this book will be particularly valuable, and especially to those who are not familiar with the specialized literature of amphibian experimental embryology.

The book begins with a short introduction summarizing the development of our ideas concerning the neural crest from its first description by Wilhelm His in 1868 to its establishment as one of the 'primary organs' of the vertebrate embryo. Particular emphasis is laid on the amphibia, since the greater part of the rest of the book is concerned with describing experimental investigations which deal predominantly with these animals. The introduction is followed by chapters dealing with the experimental analysis of the contribution made by neural crest material to the nervous system, the cranial skeleton, to the formation of pigment cells, and finally by a consideration of the neural crest as a possible inductor of such organs as the lateral line placodes and the auditory vesicle.

Each chapter contains a full but summarized account of the very numerous experimental investigations, often of great beauty and ingenuity, relevant to the problem under consideration. Probably greatest interest attaches to the account of work concerned with the contribution of the neural crest to the cranial skeleton, for it is chiefly here that the author has himself made fundamental contributions to our knowledge. It is natural that it should be considered at greater length than some other subjects. Particularly in the last chapter, dealing with the function of the neural crest as an inductor, one feels that the exigencies of time in the lecture, or of space on the printed page, have led to considerable compression. Had it been possible, one would have welcomed a fuller discussion of the significance of

the many experiments which are described, and perhaps some general summary of the results achieved. Within its inevitable limits however, the book forms not only an admirable account of a tissue of fundamental importance in the early embryo, but a fascinating introduction to the methods and possibilities of experimental embryology; it provides, in fact, a very good example of the necessity of experimental methods for any real understanding of developmental processes. The value of the book is increased by an extensive list of references up to the year 1948. F. GOLDBY

Atlas der normalen Histologie und mikroskopischen Anatomie des Menschen. By E. VON HERRATH and S. ABRAMOW. (Pp. 139; 398 figures, mostly coloured; 11·45 dollars.) Stuttgart: Georg Thieme Verlag. 1950.

This Atlas of, on the whole, excellently reproduced colour micro-photographs of histological specimens is fittingly dedicated to Dr Ernst Leitz on the occasion of the centenary of the establishment at Wetzlar of the Leitz Works.

E. von Herrath, Professor of Anatomy at Berlin, is responsible for the selection of the histological specimens which have been collected from various Institutions, and S. Abramow, of the Research Staff of Messrs Leitz, is responsible for the colour-microphotography. Authors, printers and publishers have all to be congratulated on their respective achievements. Not all the pictures are of an equally high standard, but the authors have blunted any criticism on this point by the statement that they did not want to postpone publication any longer, even though this meant the inclusion of some inferior photographs and a few black-and-white drawings.

The Atlas deals, in the usual order, with cytology, general histology and organology, and the pictures are preceded by a few pages of comments which relate to selected figures only. Some of these comments are interesting and helpful, others are of doubtful value being often too dogmatic, involved and condensed, and not always accurate.

So far as colour reproduction is concerned, the azan and iron-haematoxylin-Van Gieson preparations are the best, followed by the pictures of Pappenheim-stained blood and bone-marrow smears. Some of the haematoxylin and eosin preparations are not very good. On the other hand, a specially high standard has been reached in, for example, a transverse section of heart muscle, some silver impregnation preparations, a Nissl picture, and a frozen section of Sudan-H.-stained cartilage cells.

It is of psychological interest to note that even where the pictures are slightly blurred and would not do as black-and-white photographs, they are perfectly satisfactory in the coloured form.

The figures have lettered leader lines which are explained in the legend. In many instances these annotations are so detailed that the legend will convey a complete histological analysis of the field photographed. The fields illustrated are, in most cases, well chosen so as to be typical for the particular specimen under view, and also to include morphological entities of more general interest such as may crop up in any section. Structures which are often puzzling to the student, e.g., tangential and longitudinal sections of arterioles, are depicted and the various nuclei annotated. The differences between the various segments of the uriniferous tubule are clearly shown. Nerves and lymphatics are frequently indicated (but what are referred to as lymphatics in the adrenal medulla are not convincing).

Dynamic histology is represented with pictures from various fields.

A few points of criticism: In fig. 56 a reference should have been made to collagenous fibres. In figs. 296, 297 there is no indication of a submucosa in ureter and bladder. In fig. 110 the continuity between myofibrils and collagenous fibres is not shown convincingly. There is some inconsistency in the blood cell nomenclature used in figs. 150–184.

The Atlas is comprehensive and covers much ground, and one is thus surprised to find no representation of motor end-plates, Meissner's corpuscles, the penis and the external auditory meatus, nor are there any high-power photographs of the mucosa of the uterine

tube, the wall of a bronchus, lymph sinuses or intestinal villi. These could well have replaced some of the redundant illustrations of bone and cartilage. A more detailed index would have been helpful. There are very few typographical errors though, in fig. 254, 2nd foetal week should read month.

As would be expected in a production of this kind, the price is rather more than many students could afford, but if the volume could be made available to the student by the histology library, he would receive much help and instruction from a careful study of this Atlas.

F. JACOBY

OBITUARY NOTE

We have just heard with great regret of the death of the Editor, Professor C. M. West. A notice *In Memoriam* will appear in a later issue.

PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1950

The Annual General Meeting of the Society, for the Session 1950–51, was held in the Department of Anatomy, St Thomas's Hospital Medical School, London, S.E. 1, on Friday, 24 November 1950. The President (Prof. M. F. Lucas Keene) was in the Chair.

The following are the authors' abstracts of the papers read.

Observations on the anatomy of *Perodicticus potto*.

By D. V. Davies.
St Thomas's Hospital Medical School, London

Several skeletons have been studied and two whole specimens dissected.

Variations occur in the number of phalanges present in the diminutive index finger, possibly explaining the discrepancies in the literature concerning the arrangement of tendons to this digit. The extent to which the palatine bone participates in the formation of the medial wall of the orbit also varies, as do the articulations of the frontal bone in the region of the pterion. The position of the tympanic ring, outside the bulla, is seen in one juvenile specimen; in the others examined it is impossible to trace its subsequent history. The elongation of the spinous processes in the cervical region also affects the upper thoracic vertebrae to a varying degree. Transpedicular foramina are present in all the thoracic vertebrae except the first, and there is a tendency for similar foramina to appear in all the lumbar vertebrae also. The bar of bone completing these foramina is replaced by ligament in young specimens.

The characters of the teeth of the deciduous series are given, together with the probable order of eruption of the permanent teeth.

The arrangement of the cardiovascular system is similar to that of the Malayan slow loris (*Nycticebus tardigradus*). There is a valve in the inferior vena cava and in the portal vein, but so far no case of doubling of the inferior vena cava has been described. However, the possibility of such an occurrence cannot be excluded as the number so far examined is small.

The milk canine of the chimpanzee with special reference to that of the Australopithecinae.

By W. E. Le Gros Clark. *University of Oxford*

It has been recently stated that the milk canines of *Australopithecus africanus* and *Paranthropus robustus* do not differ in shape and dimensions, relative and absolute, from that of the chimpanzee. However, re-examination of a series of fifty immature chimpanzee skulls confirms previous conclusions that the morphological pattern of the chimpanzee milk canine does, in fact, present certain rather fundamental differences. These appear to show such clear-cut qualitative contrasts as to obviate the need for quantitative treatment, but they may, if necessary, be expressed metrically.

Congenital atlantal arch defect in the gorilla.

By A. J. E. Cave.
St Bartholomew's Hospital Medical School, London

A specimen of congenital deficiency of the posterior atlantal arch is described for the first time for the gorilla, the anomalous atlas being the sole example of its kind encountered in the examination of seventy-two gorilla spinal columns. Reference is made to the absence of record of the comparable condition for the chimpanzee and orang and to its relative frequence in the gibbon.

The axis of movement at the ankle joint and its influence upon the shape of the talus.
By C. H. Barnett and J. R. Napier. *St Thomas's Hospital Medical School, London*

The difference between the anterior and posterior transverse diameters of the trochlear surface of the talus, the so-called 'wedging' of the bone, was investigated in one hundred cartilage-covered specimens, and was found to show considerable variation.

Examination of the curvatures of the medial and lateral profiles of the trochlear surface indicates that the axis of the ankle joint is not a fixed horizontal one, but points downwards and laterally during dorsiflexion, changing abruptly as the foot passes into plantarflexion to an axis directed downwards and medially.

It was found that the mobility of the fibula, as judged by the criterion of the superior tibiofibular joint, and the amount of wedging of the talus, could be correlated with the degree of inclination of the ankle axis in dorsiflexion and plantarflexion, respectively.

Such obliquities of axis are inevitably accompanied by an outward movement of the neck of the talus as the foot moves up or down from the neutral position. This undesirable movement is neutralized by a medial rotation of the whole talus about a vertical axis, a movement which tends to bring about a loss of apposition of the adjoining articular surfaces on the talus and fibula. It is suggested, however, that this tendency is counteracted by two mechanisms. First, during dorsiflexion the fibula twists about its long axis, thus realigning its articular surface to that of the talus. Secondly, the posterior portion of the lateral surface of the talus is rounded off (thus producing 'wedging' of the bone) to enable it to maintain its intimate contact with the adjacent facet on the fibula during plantarflexion.

The essential parenchyma of the pig's placenta. By E. C. Amoroso. *Royal Veterinary College, London*

It is generally agreed that the pig's placenta is a diffuse epitheliochorial one, consisting of two epithelial layers which are closely apposed at certain points and variously separated by irregular spaces (areolae) at others. Moreover, it has been assumed that there is no destruction or degeneration of the uterine mucosa, and that the foetal membranes, all of which remain external to the uterine tissues, become apposed in a simple way to an intact endometrium. It is the purpose of the present communication to call attention to some important modifications in the two epithelial layers which constitute the essential parenchyma of the placenta of this animal.

Evidence will be presented to show that by the third week of pregnancy the uterine epithelium shows signs of degeneration and is reduced to a thin layer. A week later it again appears normal and remains uniform for a time but begins to differentiate as the endometrial ridges commence to divide; over the summits of the mucosal septa the epithelium of the inter-areolar areas is columnar while in the depths of the grooves beneath the growing tips of trophoblast the epithelium is cuboidal or flat. Opposite the areolae the uterine epithelium is likewise modified during pregnancy. The columnar cells become transformed into a symplasma and, together with disintegrating glandular epithelium, are added to the other constituents of the uterine milk.

The trophoblast remains single-layered throughout pregnancy and is, at first, columnar. After the first 3 weeks it forms a syncytium, but this is replaced on about the thirtieth day by evenly granular columnar cells. A further differentiation takes place at the time when the chorionic ridges begin to be subdivided; in the depths of the chorionic fossae between the chorionic ridges, the cells remain tall columnar and are provided with brush borders, whilst on the sides and over the summits of these ridges they become cuboidal or even flat. At numerous places near the apices of the chorionic ridges the foetal capillaries, as Tafani (1886, *Archivo della Scuola d'Anat. Pat.* **4**, 52) first showed, penetrate and run among the epithelial cells and, where the epithelium is tunnelled with capillaries, cell outlines are often lost, and the summits of the chorionic ridges are then characterized by what appears to be a vascular plasmoditrophoblast not unlike that described in the placenta of the peccary by Wislocki (1931, *J. Mammal.* **12**, 143).

Multiple foetal anomalies associated with placental hydatidiform degeneration. By J. M. Roberts. *Charing Cross Hospital Medical School, London*

A 16-week pregnancy was terminated by hysterotomy for hypertension and albuminuria on 27 April 1950, the last menstrual period having been on 22 December 1949. The conceptus consisted of a 100 mm. c.r. length foetus with a placenta showing gross hydatidiform degeneration, and it is believed that the foetus was dead before operation. The foetus showed multiple anomalies including a bilateral hare-lip, complete cleft palate, a large tumour lying beneath the left sternomastoid muscle, persistence of the right dorsal aorta, a single umbilical artery, and a poorly differentiated urogenital system associated with only partial ascent of the right kidney. The causes of these foetal anomalies may be either genetic or environmental, and because there is no previous family history of any abnormality, it is suggested that environmental and not genetic factors are probably responsible in this case. Although placental failure appears to be the most obvious cause, it is possible that its degeneration is secondary to the foetal anomalies as suggested by Hertig (1950). However, the recent work of Warkany *et al.*, in which similar multiple anomalies have been produced by maternal vitamin A deficiency, must be considered, together with other possible environmental factors.

Puncta lacrimalia and canaliculi in cyclopia. By W. R. M. Morton. *Queen's University, Belfast*

Chatterjee, in a short note in the International Anatomical Congress Abstracts, 1950, describes the presence of canaliculi and naso-lacrimal ducts in cyclops, and indicates that he is unable to accept the usual explanations of the mode of formation of these passages. His ideas are not fully elaborated in the abstract, but he appears to have overlooked Frazer's (1931) account of the normal development of the canaliculi from an area of ectoderm buried by the inner ends of the developing eyelids. Serial sections of the lower eyelids and median caruncle of a well-formed full-time human male cyclops confirm the presence of two patent puncta lacrimalia and canaliculi in this case. Their presence can best be explained by assuming that the maxillary mesoderm below each lateral half of the single eye acted as if the maxillary processes were normally situated, and grew upwards and medially forming two distinct lower lids and covering in a single central area of surface ectoderm. The puncta and canaliculi could then develop in the lines of fusion of the overlapping ectoderm, and the central region would form a single caruncle.

The metabolism of cell division in the blood cells of the chicken embryo. By R. J. O'Connor. *Westminster School of Medicine, London*

Measurements have been made of the rate of aerobic glycolysis, rate of respiration, respiratory quotient and number of dividing cells in the blood cells of the chicken embryo from the third day of incubation onward. Aerobic glycolysis could not be demonstrated and the respiratory quotient remained constant at 1·0. The rate of respiration and number of dividing cells decreased during development in such a way that a linear relationship could be established. These results are compared with similar investigations on the developing mid-brain, where the rate of respiration remained constant, but a linear relationship was established between the number of dividing cells and aerobic glycolysis.

The limbs of snakes. By A. d'A. Bellairs. *London Hospital Medical College, London*

The occurrence of rudiments of the hind limbs in certain snakes has long been recognized, but little work has been done on these structures since the beginning of the nineteenth century. Examination of serial sections of two late embryos of the snake *Trachyboa* (family: Boidae) shows the presence of a pelvis with an acetabulum, and a bone which probably

represents the femur. The latter terminates in a claw which, at least in male specimens, projects externally on either side of the cloaca. Several muscles attached to the pelvis are described and an account of the general relationships of the hind-limb skeleton is given; the pelvis is remarkable in being situated mainly internal to the ribs. The function of these limb vestiges as accessory sexual organs is discussed.

The aortic and sinus nerves of the lion and badger. By E. C. Amoroso, F. R. Bell, A. S. King and H. Rosenberg. *Royal Veterinary College, London*

Two lionesses, aged 1 and 7 years respectively, were examined for the aortic depressor and sinus nerves. One (the older) was dissected after death. The other was anaesthetized and various nerves were stimulated electrically, whilst recording arterial pressure and respiration.

The carotid sinus nerve was formed from a union, 5 mm. from the sinus, of two slender components, one from the IXth cranial nerve and the other from the superior cervical ganglion. The vaso-motor afferent fibres were found on stimulation to be located in the component from IX. The superior laryngeal nerve, which was unusually large, arose from the nodose ganglion. After approximately 2 cm. of its course it gave off a substantial branch which ran caudally parallel with the vagus; stimulation showed the absence of depressor fibres. Two other slender nerves, running with the vagus and sympathetic trunks respectively, were likewise shown to be devoid of depressor fibres, and it was concluded that in the two specimens examined there was no independent aortic nerve.

The badger, an adult female, was also examined under anaesthesia whilst recording the arterial blood pressure and respiration. A slender independent nerve which ran with the vago-sympathetic trunk in the caudal part of the neck and with the sympathetic cranially, and which in part of its course detached a fine filament which joined the vagus about 2 cm. caudal to the superior laryngeal nerve, was shown on stimulation to contain the depressor fibres. In the experimental animals stimulation was by smooth, equal induction shocks (Rosenberg, 1948) at 8·3 V. and frequency 2×126/sec.

The significance of these results is discussed.

Observations on the neural transmission of herpes and rabies viruses. By E. J. Field. *The University, Bristol*

The work of Goodpasture & Teague (1923) and Goodpasture (1925) forms the main experimental basis of the theory of axonal transmission of herpes and rabies viruses. Their results and interpretation have, however, been questioned by Marinescu & Dragonescu (1923, 1932), who claimed that invasion of the nervous system takes place through the lymphatic channels of nerves and not by the axis cylinders. Since the point is one of some importance it has been re-investigated from the morphological standpoint. Rabbits have been used throughout. They have been inoculated upon the cornea (scarification) and into the masseter muscle and sacrificed after varying intervals. The Gasserian ganglia and brain stem have been sectioned serially to determine the location of earliest lesions. The following conclusions were reached:

(1) Goodpasture's statement that inoculation into the masseter results in a characteristic mitral lesion in the corresponding trigeminal motor nucleus—a fundamental observation for the theory of axonal transmission—could not be substantiated. In fact, this nucleus may be intact when there are widespread lesions in the brain stem.

(2) No characteristic lesions were observed in the Gasserian ganglia after corneal inoculation (early cases).

(3) An early lesion usually appears on the root of the Vth nerve immediately proximal to the level at which the thick sheaths of the nerve became suddenly thinned.

(4) Both corneal and masseteric inoculation (herpes) produce a wedge-shaped necrotizing lesion which may or may not show characteristic herpetic inclusion bodies in the ventro-lateral part of the medulla, involving the ventral extremity of the spinal tract of the Vth nerve and the more superficial ventral spinocerebellar tract.

(5) Occasionally unusual findings occur, e.g. degenerative changes in Purkinje cells of the cerebellum; infiltration lesions of the nucleus ventralis of the VIIIth nerve without lesions elsewhere.

(6) The findings offer no direct support to the theory of axonal transmission. Alternative hypotheses are indicated.

The effect of muscle devascularization on motor end-plates. By D. B. Allbrook and J. T. Aitken. *University College, London*

All visible blood vessels were removed from the tibialis anterior muscle in the rabbit without damaging the muscle nerves.

Devascularization was confirmed by an intravenous injection of bromo-phenol-blue and by performing interim biopsy.

At intervals up to 100 days from devascularization, the muscles were stimulated and examined histologically, using a modified Bielschowsky-Gros silver method.

Early changes were found in the appearance and electrical reactions of the muscles, and many motor end-plates were swollen. Disorganization of parts of the muscle most severely affected followed, with disappearance of motor end-plates and necrosis of axons and muscle fibres.

Twenty-eight days after devascularization the muscles had regained normal naked-eye appearances and responded to indirect stimulation. Histologically, the nerves were seen to have regenerated, though the pattern of re-innervation was irregular. Where the muscle fibres had not degenerated the nerves followed the old neurilemmal sheaths, but in areas where the muscle had regenerated the nerve fibres were fine and varicose, and many irregular motor end-plates were found.

Observations on the human sternum. By G. T. Ashley. *The University, Manchester*

This is a record of observations made on one hundred East African sterna, by permission of Prof. Galloway of the University College of East Africa, and is concerned with their size, shape and sexual differences.

The dried bones were inspected, measured and X-rayed.

The following conclusions were arrived at:

(1) The variation in shape of the body of the sternum permits classification of sterna into three types: (I) body narrow and with parallel sides; (II) body narrow at top and broad below; (III) body broad and with parallel sides.

(2) There would seem to be a relationship between the definitive shape of the body of the sternum and the number of ossification centres from which it arose.

(3) The ratio of manubrial length to body length is not by any means a sure method of determining the sex of the sternum.

Electromyographic study of the action of the serratus anterior muscle in respiration. By W. T. Catton and J. E. Gray. *King's College, Newcastle upon Tyne*

There is a general agreement in standard text-books of anatomy that the serratus anterior muscle can play a part in inspiration, although modern authorities differ in their assessment of the importance of the muscle in respiration in man.

The electrical state of the muscle has been estimated in normal subjects, and in one case of paralysis of the serratus anterior. Surface electrodes were used, and potentials were recorded by means of a cathode-ray oscillograph. Electromyographic study failed to demonstrate any activity in the serratus anterior muscle during voluntary deep breathing, breathing obstructed by forcing the subject to breathe through a narrow tube, or coughing.

Massive responses were obtained from the muscle when the arm was raised from the side.

The structure of human gastric arterio-venous anastomoses.
By T. E. Barlow. *King's College, Newcastle upon Tyne*

Schumacher (1938) describes two types of arterio-venous anastomoses—the first a complicated knot of vessels (glomus), the walls of which are characterized by an epithelioid type of cell in the tunica media which replaces the typical muscle cell and is said to control the anastomosis by swelling or shrinking. The other type exists as a sinuous but more direct connexion between artery and vein, and may be controlled by additional plain muscle cells in the tunica media. Direct arterio-venous anastomoses in the rabbit's ear, described by Clark & Clark, are also said to be controlled by a thick muscular wall.

Human gastric arterio-venous anastomoses of the direct type have already been described in the submucous layer and now subjected to serial section throughout their entire length. Contrary to expectation there did not appear to be any indication of increased musculature in the tunica media throughout the course of the vessel, but simply a direct and gradual change-over from an arterial to a venous type of structure. Sections through a second specimen, however, showed thickened walls and projections of cells into the lumen. It is suggested, therefore, that these anastomoses are controlled in typical fashion and that the first specimen was fixed when the anastomotic channel was open.

The arterial supply of the urinary bladder. By J. L. Braithwaite. *The University, Manchester*

Modern text-books of anatomy are deficient in the description of vesical vascularization, and this problem has been re-investigated. Fifty infant bladders have been injected and dissected, using several techniques; in addition thirty adult pelves have been dissected.

In both sexes the greatest vesical area is supplied by the superior vesical arteries, by branches arising as two or three trunks from the umbilical arteries. The inferior vesical artery in the male, homologous with the vaginal artery in the female, supplies the infero-lateral aspects of the bladder and bladder neck. The vesiculo-deferential artery in the male corresponds to the uterine in the female, supplying branches to the terminal part of the ureter and a variable amount of the posterior aspect of the bladder, including the trigone. Of the branches from other vessels, the middle vesical, the obturator and the inferior epigastric are the most important.

FEBRUARY 1951

An ordinary meeting of the Society, for the Session 1950–51, was held on Friday, 16 February 1951, in the Department of Anatomy, Guy's Hospital Medical School, London Bridge, London, S.E. 1. The President (Prof. M. F. Lucas Keene) was in the Chair.

The following are the authors' abstracts of the papers read.

Sesamoid bones of the hand. By J. Joseph. *Guy's Hospital Medical School, London*

The radiographs of the hands of the adults and children which were used for a previous communication in which the interphalangeal sesamoid of the thumb was considered in some detail, were studied with regard to the rest of the sesamoids of the hand. It was found that both metacarpo-phalangeal sesamoids of the thumb appear before the interphalangeal sesamoid of that digit, that they appear earlier in girls than in boys and that the time of their appearance varies in different individuals. These sesamoids appear in all adults.

Regarding the other metacarpo-phalangeal sesamoids, most individuals could be arranged in three larger and two smaller groups: (*a*) those with none, (*b*) those with bilateral sesamoids at the 5th metacarpo-phalangeal joint, (*c*) those with bilateral sesamoids at both the 2nd and 5th metacarpo-phalangeal joints, (*d*) those with a unilateral sesamoid at the 2nd or 5th metacarpo-phalangeal joint, (*e*) those with bilateral sesamoids at the 5th and a unilateral sesamoid at the 2nd metacarpo-phalangeal joint. The actual incidence of the 5th metacarpo-phalangeal sesamoid was 70 % and of the 2nd, 35 %.

Study of the radiographs confirms the view that the sesamoids of the hand are true skeletal elements since: (*a*) they ossify in a certain order, (*b*) there is the usual sex difference in their time of appearance, (*c*) there are individual variations in their time of appearance, (*d*) they are usually bilateral, (*e*) their appearance cannot be related to any known external environmental factors such as pressure or muscular power. On the other hand, although the majority of subjects can be arranged in groups, there appears to be a large number of different groupings, if everybody in the sample is included. These variations may be due to the fact that, from an evolutionary point of view, these bones are disappearing.

Schwann cell activity in tissue culture of two degenerating nerves of different fibre spectrum. By G. A. Thomas. (Introduced by J. Whillis, *Guy's Hospital Medical School, London*)

Explants of degenerating rabbit nerves of very different 'fibre spectrum' viz. the nerve to the median head of the gastrocnemius muscle, a nerve with very large fibres and the sural nerve, a nerve with small fibres, have been grown in tissue culture. The number of Schwann cells which migrated from the explants during 3 days of incubation has been estimated.

The number of Schwann cells which migrated per nucleus in a transverse section of the nerve at 5, 10 and 15 days of degeneration is greater in the large-fibred nerve to the median head of the gastrocnemius than in the small-fibred sural nerve.

It appears, therefore, that nerves of different 'fibre spectrum' show a quantitative difference in Schwann cell migration during Wallerian degeneration. This difference may possibly be due either to the different amounts of breakdown products of the myelin sheaths which may act as a stimulus to migration in the two nerves, or to differences in the space within the Schwann tubes allowing greater freedom of movement to the Schwann cells in the nerve to the median head of the gastrocnemius and less freedom in the sural nerve.

The mechanism of suckling. By E. Gwynne Evans. (Introduced by J. Whillis, *Guy's Hospital Medical School, London*)

Sucking and suckling are not synonymous terms. Sucking is an acquired function of the oro-facial muscles, and is brought about by the creation of intra-oral negative pressure. It is an intermittent process interrupted by acts of swallowing during which respiration is inhibited. Suckling is an inborn function of the oro-facial muscles, and is brought about by a pumping and squirting action of the soft palate, tongue and jaw muscles. It is a rhythmic process during which respiration continues without interruption.

Extracted frames from films of the movements of the oro-facial muscles in sucking and suckling were shown.

The function of the plantar aponeurosis. By J. H. Hicks. (Introduced by R. G. Harrison, *University of Liverpool*)

Experiments performed on freshly amputated legs demonstrate that, of the various current descriptions of the mechanical structure of the foot, the one which likens it to a truss is nearest to the truth.

A truss is a triangular structure composed of two rods and a tie. In the foot the tie is the plantar aponeurosis, and there are five trusses radiating from a common posterior rod. It has been confirmed that the plantar aponeurosis resists the deforming effect of body weight, and that in the absence of the plantar aponeurosis the foot loses its normal shape and function.

These experiments have shown that the height of the truss varies with each step in walking, and explain the mechanism whereby this is effected. The triangle (the truss) becomes taller when its base (the plantar aponeurosis) is made shorter. The shortening of the plantar aponeurosis is brought about by a windlass mechanism at its anterior end. The drum

of the windlass is the metatarsal head; the cable which is wound around the drum is the plantar ligament of the metatarso-phalangeal joint (to which the plantar aponeurosis is shown to be strongly attached); and the handle which winds up the cable is the proximal phalanx.

The activity of leg and foot muscles in standing.
By J. W. Smith. *University of St Andrews*

The importance of muscles as a factor in maintaining the longitudinal arch of the foot in standing would appear, from a survey of current literature, to be a matter of controversy.

In this investigation, an attempt has been made, by the study of electromyographic records, to assess the activity of the muscles of the leg and foot while the subject is standing in a relaxed attitude. The results indicate that the muscles probably play a minor part in maintaining the arches of the foot in these circumstances.

Quantitative observations of certain muscles in the rhesus monkey.
By R. E. M. Bowden. *Royal Free Hospital School of Medicine, London*

The data presented in this communication form the preliminary stage of an investigation of changes in voluntary muscle due to poliomyelitis. The sitting height and body weight of 203 rhesus monkeys were measured and plotted, and regression lines were calculated for three groups of animals in good, fair or poor states of nutrition. As might be expected, the better the state of nutrition, the higher the weight tended to be for a given sitting height. The sex differences were analysed.

The right and left m. sartorius were weighed in eighty-six animals. There was some correlation between muscle weight and sitting height, but there was closer correlation between muscle weight and body weight. The curve was calculated using the formula $y = a + b \log x$ in which y = weight of muscle, and x = body weight. No significant difference was found in curves computed for males and females. It was necessary to see if there was any evidence of left or right sided dominance of the limbs, and muscle weight was used to investigate this problem. There was no evidence of such dominance in the group as a whole, although it could not be excluded for the individual.

The mean diameter of muscle fibres in m. gracilis was measured in seventeen muscles; considerable variation was found between these means, but there was no evidence of correlation between the muscle fibre diameter and the body weight. This raises several problems of interest.

Graphic representation of efficiency in stereognosis. By J. Whillis. *Guy's Hospital Medical School, London*

A method was demonstrated of representing graphically efficiency in stereognosis. The apparatus used is a tapering Perspex rod the thickness of which is estimated by the subject with his eyes closed. With the eyes open he is then asked to select on the rod the original point he touched and the error, if any, is measured.

Gustatory enzymes and appreciation of taste. By Abdel Fattah el Baradi and Geoffrey H. Bourne. *London Hospital Medical College, London*

It has been shown by one of us (G.H.B.) that a phosphatase which splits glycerophosphate is localized in taste-buds or in the epithelium adjacent to taste-buds in various species of mammals.

In the papilla foliata of the rabbit this enzyme is localized in the epithelium lining the papilla. If other substrates such as adenosinetriphosphate, hexose diphosphate and muscle adenylic acid are used in place of glycerophosphate the reaction is still localized in the same sites. This indicates either that the phosphatases splitting the various substrates have an identical localization or that the one enzyme is capable of splitting a variety of substrates.

In addition to the phosphatases a simple esterase has been found by histochemical means

to be present in the same region. There is only a slight concentration in the epithelium, but the taste-buds themselves gave a strong positive reaction for the enzyme. The accessory serous salivary tissue also gave a strong reaction.

The occurrence of various enzymes in this site suggested that they might be concerned with the chemical mechanism of taste. As a preliminary investigation of this possibility a number of substances were added to the substrate in which the tissue was incubated, and in which the reaction between enzyme and substrate takes place. At first the four basic taste substances were investigated, i.e. quinine (bitter), sodium chloride (salty), hydrochloric acid (sour) and sucrose (sweet). The acid, in virtue of its effect in altering the pH of the reaction mixture, had the expected effect of inhibiting the action of all the enzymes. Sugar and sodium chloride had no effect on any of the enzymes but quinine almost completely inhibited the esterase. Other strongly tasting substances were tested such as vanillin, capsicum, aniseed, peppermint, saccharine and also infusion of tea and coffee.

Some of these substances inhibited the phosphatases and some the esterases, some affected both and some affected neither.

From these preliminary results it seems that inhibition of one or more enzymes or enzyme systems in or adjacent to the taste-buds may be part of the chemical mechanism of taste.

Comparative studies of vertebrate electrocardiograms. By Francis Davies and E. T. B. Francis. *University of Sheffield*

An electrocardiogram of the Nile crocodile, when compared with the normal human electrocardiogram, revealed significant differences which can be correlated with the absence of any specialized conducting tissue in the reptilian heart (as reported previously by the present authors). A comparative survey has now been made of electrocardiograms of other vertebrates, especially mammals and birds, e.g. dog, cat, rabbit, rat, mouse, horse, sheep, cow, fowl and pigeon, and it has been possible to relate the time taken for the cardiac impulse to spread through the ventricles (QRS complex) to both the size of the heart and the degree of histological specialization attained by the conducting tissue.

Gland-like structures in the renal pelvis and calyces of the human kidney. By F. R. Johnson. *University of Sheffield*

There are numerous references in the literature to gland-like structures in the pelvis of the ureter and in the ureter itself. These are usually described as being present only in pathological specimens. Examination of serially sectioned major and minor calyces and pelvis of normal human kidneys has shown that these structures are common and the preparations show no evidence of pathological processes. The gland-like structures are of two main types; first, those in which the epithelium has become nipped off by strands of connective tissue from the substantia propria, and secondly, those which appear to be produced by a burrowing of the complete layer of epithelium into the underlying connective tissue..

The development of the superficial and the deep glands of the oesophagus. By B. A. E. Johns. *University of Sheffield*

The appearances of the oesophageal epithelium of the foetus was described at various stages. The indifferent epithelium of the early foetus is seen to differentiate in two directions: (1) into a stratified ciliated epithelium, and (2) into a columnar epithelium, the cells of which are remarkably clear and tall. The latter is seen at the end of the oesophagus and progressively diminishes in extent with advancing foetal age. From it the superficial cardiac oesophageal glands are seen to develop, whereas the ciliated epithelium becomes converted into stratified squamous epithelium. Islets of ciliated epithelium may however remain up to the time of birth. The development of the deep, submucous glands is also described.

Motor localization in the cat's spinal cord. By G. J. Romanes.
University of Edinburgh

Retrograde changes in the ventral horn cells of the lumbo-sacral spinal cord have been studied after crushing peripheral nerves in the lower limb on more than one occasion.

The number and position of the large cells which react to injuries of this type are remarkably constant. It is found that the cells lying in groups (essentially two in number) at the lateral border of the ventral horn are altered by denervation of muscles supplied by dorsal divisions of the ventral primary rami. Those more medially situated react to injury of the ventral divisions. The latter cells show five completely separate groupings or columns each of which contains cells whose processes innervate the muscles producing movements at one or two adjacent joints of the limb; e.g. hip, hip and knee, knee and ankle, ankle and toes or toes only. Of these the most proximal muscles are innervated by the most ventral group and the distal by the most dorsal group.

Within any one column the cells supplying a given muscle are mixed with those innervating other muscles of the same group, though there is a tendency for some of the larger muscles to be supplied by subdivisions of one column, e.g. the hamstrings.

The position and longitudinal extent of cells supplying individual muscles have been plotted and related to pre- or post-fixation in the plexus. In extreme degrees of prefixation the cells supplying any given muscle may lie almost a segment higher in the spinal cord than in animals with post-fixed plexuses, but the relation of the various groups of cells to each other remains constant.

Nervous pathways in the developing human labyrinth. By C. C. D. Shute.
Middlesex Hospital Medical School, London

In a number of human embryos fixed and stained by de Castro's silver impregnation method and sectioned serially, a study has been made of the paths by which the peripheral innervation reaches the sensory epithelium of the otic labyrinth.

The precise route followed by fibres of the nerves to the ampullary cristae is subject to variation. On one side in a 48 mm. embryo, half the innervation of the superior crista runs a short distance through the developing facial canal, re-entering the inner ear by a separate channel through the cartilaginous capsule.

The common ampullary nerve to the superior and lateral cristae, as it winds round the ventral end of the utricle, normally gives off a few fibres to the adjacent part of the utricular macula. This accessory innervation of the utricle is comparable developmentally with that of the saccule by the branch of the utricular nerve associated with the name of Voit (1907).

In a 30 mm. embryo, peripheral processes of inferior vestibular ganglion cells run to the junctional region between the saccular macula and the commencement of the cochlear duct. At later stages vestibular fibres can be followed into the base of the spiral canal, and thence by a recurrent course to the caudal end of the saccular macula. This is believed to be the true nature of the so-called cochleo-saccular nerve, which Hardy (1934) supposed to arise from cochlear ganglion cells.

Ganglion cells migrate to the basal coil of the cochlear duct from the region where the original cochlear and inferior vestibular cell masses fuse. Intermediately placed ganglion cells occur as a relic of this process, and the nerve fibres derived from them form an apparent vestibulo-cochlear anastomosis which may be identified with that described by Oort (1918). A central group of ganglion cells is also found on the course of the facial nerve marking the site of migration of geniculate cells from the superior vestibular region.

Connexions of lumbar 'intermediate' sympathetic ganglia. By P. A. G. Monro. *London Hospital Medical College, London*

Material obtained post mortem from a patient who had undergone a paravertebral sympathectomy (Right, T8–L3; Left, T4–L3) has revealed the presence of 'intermediate' sympathetic ganglia associated with the nerve roots of the first four lumbar nerves on both sides. The electrical skin-resistance method had earlier revealed the sudomotor function

pattern which indicates the peripheral connexions of these ganglia. Dissection of the nerves and their rami communicantes on the left side has been followed by block impregnation with silver in an attempt to follow connexions with the viscera. It appears that the ganglia are associated with both 'grey' and 'white' rami of the 1st and 2nd lumbar nerves. Many ganglionic cells on the 'white' rami show degenerative changes—presumably because their post-ganglionic fibres have been sectioned by the paravertebral sympathectomy, but most of the cells in the ganglia at the base of the 'grey' rami appear normal and their rami may be traced along the course of the lumbar arteries towards the aorta and have therefore escaped section. Such a pathway may also contain preganglionic fibres to the cells of the mesenteric ganglia. It seems probable, therefore, that thoraco-lumbar paravertebral sympathectomy may not be sufficient to denervate the viscera; it almost invariably fails to denervate the upper lumbar dermatomes.

Observations on the mesodermal condensations in the vicinity of human tooth germs. By C. H. Tonge. *King's College, Newcastle upon Tyne*

The literature refers to the dental papilla and the dental follicle as mesodermal condensations related to the tooth germ. The object of this study was to examine the mesodermal condensations at early stages in development in view of the paucity of descriptions prior to the formation of the dental papilla and dental follicle.

A generalized mesodermal proliferation exists beneath the ectoderm of the tooth bearing area in 15–16 mm. embryos.

At the 28 and 44 mm. stages the mesodermal condensation forming the periosteum of the jaw bones reaches the deepest portion of the lip furrow sulcus and is continued around the ectodermal portion of the tooth germ.

Progressive invagination of the mesoderm within the growing and differentiating enamel results, in the 60 and 100 mm. embryos, in a separation of the mesodermal sheet into three parts:

(*a*) The periosteum of the jaw bones reaching the oral epithelium.
(*b*) The dental papilla.
(*c*) The dental follicle formed around the tooth germ.

Later developmental stages complete the formation of the mesodermal tissues of the tooth and its supporting structures from these three defined condensations.

A specimen of synotia in the sheep. By J. W. Millen and J. Davies. *University of Cambridge*

Gross aberrations in the development of the mandibular arch are uncommon in the human but are not infrequent in the sheep. The specimen of synotia which is the subject of the communication was obtained during the routine collection of foetal material.

External examination of the specimen showed apposition of the pinnae in the midline anteriorly and complete absence of the eyes, nose and mouth. Serial sections of the head were cut and examined. The histological appearances was described and their possible interpretation discussed.

An anatomical analysis of 700 cases of hernia in East Africans. By G. T. Ashley. *University of Manchester*

The work to be reported is to some extent surgical, but it raises anatomical questions which call for further investigation.

Through the courtesy of the D.M.S. Uganda and the staff of Mulago Hospital, the author was permitted to investigate the case histories of patients operated on for hernia in the hospital, and to perform personally a number of herniotomies. During 1948 and 1949, 700 such operations were performed including eighty-six by the author and a number under the author's supervision.

It was found that:

(1) The relative incidence of inguinal to femoral etc., male to female, was comparable to findings in Europeans and Americans.

(2) Direct inguinal hernia appears to be abnormally common amongst Africans. There were ten direct herniae for every eleven indirect as compared with recent American figures of four direct for every twenty-one indirect.

(3) Both intra- and extra-saccular herniae of large bowel and of bladder were also abnormally common.

(4) Certain tribes, e.g. the Baganda, appear to be much more susceptible to hernia than other tribes, e.g. the Banyaruanda. From available figures the Banyaruanda would seem to be almost immune to hernia. However, there are certain factors which indicate that this relative immunity may be more apparent than real.

The points raised at (2), (3) and (4) above indicate that an extensive field investigation into the anatomy of the inguinal region in certain East African tribes would be both interesting and instructive.

Some preliminary observations upon the Gram-positive cells of the human anterior pituitary. By C. L. Foster and R. R. Wilson. *St Mary's Hospital Medical School, London*

Formalin fixed sections of human pituitary, stained with crystal violet, and subsequently treated with iodine and differentiated in clove oil according to the technique of Gram, show a strong positive reaction in the granules of the basophils.

Following the methods used by Stacey and his colleagues who were investigating the Gram reaction of certain micro-organisms, it was found that the staining capacity of the pituitary cells could be partially or completely destroyed by a preliminary treatment of the sections with hot bile salt solution. This treatment also removes, as does crystalline ribonuclease, the pyronin basophilia of the cytoplasm of pituitary cells.

Stacey was able not only to convert Gram-positive organisms into Gram-negative ones by bile salt extraction and ribonuclease digestion, but succeeded in converting the bile extracted Gram-negative cyto-skeletons into Gram-positive ones by replating with magnesium ribonucleate. From these results, coupled with chemical analysis of the bile extract, Stacey concluded that the Gram-reaction was associated with the presence of nucleic acids in the cyto-skeleton.

In the pituitary, however, it was found that although ribonuclease readily destroyed the cytoplasmic basophilia of the cells, prolonged incubation in the enzyme was without any appreciable effect upon the capacity of the basophils to react to Gram's stain.

If it is assumed that the Gram reaction of some micro-organisms and basophil granules is indicative of certain common properties, then the results so far obtained suggest that the reaction of the basophil cells is due to something more than the presence of ribonucleic acids.

APRIL 1951

An ordinary meeting of the Society for the Session 1950–51 was held on Friday, 13 April 1951, at the Royal Free Hospital School of Medicine, London, W.C. 1. The President (Prof. M. F. Lucas Keene) was in the Chair.

The following are the authors' abstracts of the papers read.

A comparative study of the rate of atrophy of skeletal muscle following anterior ramisection and acute anterior poliomyelitis in the rhesus monkey. By R. E. M. Bowden. *Royal Free Hospital School of Medicine, London*

A comparison of the rates of atrophy of skeletal muscle after anterior ramisection and acute anterior poliomyelitis seemed useful, for any striking differences might shed more light on the disease process in the muscles of infected animals, and a knowledge of the degree and time course of atrophy is essential if a critical assessment of various forms of physical treatment is to be made.

Unilateral anterior ramisections were performed in eleven adolescent animals, and the animals were sacrificed at intervals ranging from 7 to 170 days after operation. Mm. sartorius,

gastrocnemius and tibialis anterior of both sides were excised and weighed. The weight of a paralysed muscle was expressed as a percentage of the weight of the contralateral normal muscle, and this percentage value (y) was plotted against the time since operation (x). For each of the three muscles the points were found to lie around curves computed by the formula $y = K + 10^{a+bx}$, where K represents the non-muscle-cell phase of the muscle.

The rate of atrophy of m. sartorius in poliomyelitis was studied in forty adolescent animals with *clinically complete* paralysis of the hip flexors and the quadriceps group. The durations of complete paralysis ranged from 1 to 164 days. Since the nature of the disease and distribution of paralysis make it impossible to have controls in the same individual, the weight of the m. sartorius at the time of inoculation of the virus was estimated by reference to data obtained in a series of healthy animals. The weight of the paralysed muscle was expressed as a percentage of the estimated normal weight and this value was plotted against the time of duration of paralysis. The results suggest that the rate of atrophy after anterior poliomyelitis may be more rapid than after anterior ramisection. The final degree of atrophy does not appear to differ significantly.

Histological changes in the liver of the adult rat in tryptophan deficiency. By A. S. Cole and P. P. Scott. *University of Bristol and Royal Free Hospital School of Medicine, London*

Feeding experiments, using a diet deficient in the essential amino-acid tryptophan, have previously been carried out on young animals. In the experiments reported below adult rats were used.

The rats were fed a tryptophan-deficient diet for periods of from 85 to 153 days, during which they lost half their original body weight and became thin, listless and anorectic. The diet contained 14·7 % acid hydrolysed casein and 0·3 % cystine and was adequate in other respects. The livers of the rats showed shrunken vacuolated parenchymal cells with poor affinity for acidic dyes, normal glycogen content, and histologically visible fat which had a periportal distribution. Cytoplasmic basophil bodies were not discernible and the ratio of nucleolar to nuclear volume was high.

To determine whether these changes were the result of inanition, rather than of tryptophan deficiency, pair-fed control rats were given a diet containing whole casein, in place of the hydrolysate, in amounts restricted in weight to that consumed by the deficient rats. They lost about two-fifths of their original body weight and were thin, active, ferocious and ravenously hungry. The liver parenchymal cells were small with intensely eosinophilic cytoplasm, almost complete absence of glycogen, no visible fat, but traces of basophil cytoplasmic bodies. The nucleoli were somewhat enlarged.

Rats fed the whole casein diet *ad lib.* for comparison were healthy and maintained their weight. Their large, slightly eosinophilic parenchymal cells contained glycogen, some visible fat, prominent basophilic cytoplasmic bodies and normal nucleoli.

Little difference was apparent between animals fed the totally deficient diet and others given a supplement of 0·01 % L-tryptophan per 100 g. diet, although those receiving 0·2 % tryptophan were healthy and comparable with rats receiving whole casein *ad lib.*

When 0·2 % tryptophan was added to the deficient diet or whole casein substituted for the hydrolysate, deficient rats recovered most of the weight lost during the depletion period, but their livers became intensely fatty.

Lack of tryptophan seems to cause derangement of fat metabolism in liver cells and may also affect ribonucleoprotein and carbohydrate metabolism.

The posterior wall of the inguinal canal with particular reference to the cremaster muscle and cremasteric fascia. By M. J. Blunt. *Royal Free Hospital School of Medicine, London*

The spermatic cord and round ligament are often considered as lying free in the inguinal canal, but in all of sixty canals, forty-four male and sixteen female, which have been dissected the cremaster and cremasteric fascia have shown an attachment to the posterior

wall of the canal. It is thought that they provide a fascial reinforcement to the posterior wall of the canal and that the muscular fibres which are attached close to the deep inguinal ring are of functional significance.

With regard to the attachments of the lower fleshy fibres of the internal oblique and transversus abdominis, Chandler's (1950) findings have been confirmed.

The deep inguinal ring was always found to be a slit-shaped opening except when it was occupied by a hernia or a large peritoneal dimple. The resemblance to a fibrous sling noted by Lytle (1945) was not observed in any of the canals dissected.

Dimpling of the peritoneum into the deep inguinal ring has been found in thirteen out of the sixty canals dissected, in five other canals there was an oblique inguinal hernia. Fitch (1931) noted this dimpling in 10% of the canals examined by him.

The reflected part of the inguinal ligament has been found in all but eight canals. In these eight there was insufficient aponeurotic thickening to justify designation as a separate ligament. This is at variance with Chandler's findings; he described a reflected part of the inguinal ligament in only three out of 290 canals. It is proposed to continue observations over a larger series.

Observations on the blood supply of the lesser curvature of the human stomach. By T. E. Barlow. *King's College, Newcastle upon Tyne*

It is commonly stated that the blood supply of the lesser curvature is less than that elsewhere in the gastric mucosa. This observation is based on the study of the mucosa separated from the musculature in which the vascular plexus of the lesser curve appears to be much less rich than elsewhere. It is the purpose of this study to point out that the difference in blood supply is much more apparent than real, and that the number of mucosal arteries per unit area of the lesser curvature is approximately the same as for any other part of the gastric mucosa.

Coronary arteries in the camelidae. By W. C. Osman Hill. *Zoological Society of London*

The distribution of the coronary arteries, with special reference to the blood supply of the atria, was discussed on the basis of dissections of hearts of *Camelus dromedarius* and *Llama huanachos*. Comparison was made with the author's previous results in *Elephus maximus*. The hearts were demonstrated at the meeting.

Notes on the development of the mandibular arch, with special reference to congenital fistulae of the lower lip. By J. C. Warbrick. *University of Glasgow*

Models were made of the mandibular arch in a series of embryos, ranging from 5 to 16 mm. The appearance of the ventral aspect of the mandibular arch in each embryo is described. The implications of these findings in relation to the congenital fistulae of the lower lip are discussed, and compared with the conclusions of Sicher & Pohl (1934).

Placentation in Père David's Deer, *Elaphurus davidianus*. By R. J. Harrison and W. J. Hamilton. *Charing Cross Hospital Medical School, London*

Recently His Grace the Duke of Bedford generously made available four specimens of the genital tract of this deer with the contained foetuses. Before fixation three of the specimens were injected with coloured materials, two through the foetal, the other through the maternal vessels.

The gravid uterus is in the form of a sphere, it being difficult to determine the position of the non-pregnant horn. On opening the uterus, an oblique septum is seen partially dividing the uterine cavity. The relationship to the septum of the oligo-cotyledonary placenta, the chorion, amnion, allantois and umbilical vessels is similar in all four specimens. The cotyledons are five in number in three of the specimens, the fewest so far described in the Cervidae (Andresen, 1927), and are large, ovoid and convex. The foetal villi are straight

filiform processes, several arising from a common stem, and they interlock in the spaces made by the network of maternal crypts. The blood supply of the placenta is described from the injected specimens; intra-epithelial capillaries are common in the foetal epithelium. The maternal tissues are apparently everywhere covered by epithelium in which giant cells are occasionally present. The structure of the foetal villi varies in different places.

Haemoglobin formation in the chicken embryo. By R. J. O'Connor. *Westminster School of Medicine, London*

The haemoglobin content of the circulating red blood cells of the chicken embryo has been measured from the third day of incubation onwards. The amount of haemoglobin per cell increases until the seventh day and then decreases. However, when by measuring the cell volume haemoglobin is expressed as a concentration in red cells, there is a rise of concentration which is rapid until about the eighth day and slow for the remainder of incubation. With these variations there are variations in the rates of respiration and cell division which correspond sufficiently to suggest relationships to haemoglobin formation.

Congenital urethral atresia and associated abnormalities in a still-born infant. By B. Morson. (Introduced by E. W. Walls, *Middlesex Hospital Medical School, London*)

Birth of the infant was obstructed by a large and protuberant abdomen into which a stab incision had to be made for delivery to be completed. The infant showed maceration and had a greatly enlarged abdomen through which a large mass could be felt. The head, neck and thorax with their contents showed no abnormality. The abdomen was opened by a mid-line incision, when the following condition was found:

The alimentary canal appeared normal down to the ileo-caecal junction. The caecum and appendix were, however, foetal in type and position, and the ascending colon still retained a mesentery. Two and a half inches from the ileo-caecal junction the lower bowel was replaced by a large thick-walled sac 4½ in. long and in places 6 in. across. But for some shiny green mucus the walls of the sac were in apposition. About 1 in. from its distal limit this hindgut sac communicated with the bladder anteriorly by an opening half an inch in diameter. It had no other outlet. The bladder was very thick walled and contained no urine. The urachus and lateral umbilical vessels were normal, and the ureteric orifices at the base of the bladder were patent. At the base of the bladder and close to the opening into the hindgut there was a pin-point depression which presumably represented a closed internal urethral meatus. From here a fibrous cord passed down into the pelvis for an inch before dividing into two; one division passed down to reach the region of the tip of the coccyx, where it fused with a blind pit half an inch deep, lined by skin, the other passed in the direction of the penis and became fused with a penile urethra which extended for three-quarters of an inch from the external urethra meatus. The right testis was quite atrophic and placed within the abdomen. The placenta and umbilical cord appeared normal.

Elements of foetal prostate were found in sections of the fibrous cord near the base of the bladder but no definite lumen with an epithelial lining could be demonstrated in this situation or in either of the divisions described above.

Sections of bladder and of hindgut showed great hypertrophy of the muscle layers, but in each case the epithelium was of normal type.

The alkaline phosphatase reaction in the suprarenal and the kidney. By J. M. Yoffey. *University of Bristol*

During the course of some histochemical work on the suprarenal cortex, the alkaline phosphatase content of this tissue was investigated. The reticularis appears at first to be the zone richest in phosphatase, but as the phosphatase is mainly nuclear, and the cells of the reticularis possess less cytoplasm than those of the fasciculata, this appearance may be illusory. The medulla is practically devoid of phosphatase, and there is a sharp cortico-

medullary junction. Incubation for periods up to 48 hr. showed little evidence of phosphatase diffusion. The rat suprarenal, containing a central phosphatase-negative medulla surrounded by a phosphatase-positive cortex, would seem to be admirable for investigating the diffusion of alkaline phosphatase, which could spread from all sides centripetally into the medulla. Little evidence of such diffusion, however, has so far been found in this situation.

By way of contrast, the kidney of the tigress contained glomeruli which yielded an intensely positive alkaline phosphatase reaction. No evidence was found of the centrifugal diffusion of phosphatase from these glomeruli, and there was no obvious diffusion gradient.

Histochemical changes produced in the adrenal cortex of the male adult rat by the action of anaesthetic drugs. By FRANCES B. ROBINSON. *University of Bristol*

An investigation was carried out of some of the effects of anaesthetic drugs on the histochemical picture of the adrenal cortex of 618 isolated male adult rats. The anaesthetic drugs used included general and local anaesthetics, narcotics, hypnotics and sedatives, and an attempt was made to determine whether they acted on the adrenal cortex as specific depressants, as they do on the central nervous system, or as non-specific stress stimuli producing an alarm reaction.

The results indicate that anaesthetics do act on the adrenal cortex as non-specific stress stimuli. Further work is required on hypophysectomized rats to determine whether this action is direct, or indirect via the anterior lobe of the pituitary.

It was found possible to demonstrate histochemically the three stages of Seyle's adaptation syndrome following the administration of anaesthetic drugs over varying periods of time.

Observations on the origin and development of the hairless palmar and digital epidermis. By N. CAUNA. *King's College, Newcastle upon Tyne*

Serial sections and reconstructions of twenty-one human embryos from full term to 7 mm. stages have been studied. The hairless epidermis can be identified and its distribution traced to the earliest stages of limb formation.

In the fully developed hand the hairless epidermis covers the palmar surface of the hand and digits, and also the dorsal surface of the distal phalanges. This condition is found in all specimens between 28 mm. and full term. Prior to the 28 mm. stage the area of hairless epidermis becomes progressively more extensive the younger the embryo. At 16 mm. it covers the palmar surface of the hand and digits extending dorsally to the middle of the proximal phalanges. At 12 mm. the future hairless epidermis covers the ventral surface of the entire limb and extends on to the body wall ventrally and caudally where it is continuous with the epidermis of the lower limb. Dorsally it covers the entire back of the hand.

At 7 mm. the entire limb bud is covered by thickened ectoderm extending to the body wall around the limb root, except dorsally. Caudally the thickening is continuous with that of the lower limb along the Wolffian ridge; cranially it blends with the placodal area of the head. The future digital area carries a ridge consisting of high columnar ectoderm like that of the head placodes. This 'limb placode' is the first differentiation in the development of the limb, appearing simultaneously with the head placodes.

As an explanation of the above findings, the suggestion is made that the hairless epidermis of the hands represents a primitive touch organ, and that during evolution the body wall has been modified into a projection to carry this 'placode'. The developmental rotation of the limb bud may indicate an adaptation of the 'tentacle' to additional functions (locomotion, etc.) in evolution.

Certain aspects of the non-cortical telencephalic centres of *Galago moholi*. By H. M. DAITZ. (Introduced by E. W. Walls, *Middlesex Hospital Medical School, London*)

After formalin fixation four specimens of the brain of *G. moholi* were sectioned serially in three different planes at thicknesses ranging from 10 to 40μ, and stained for Nissl's substance and for myelin.

The non-cortical telencephalic centres examined were: the olfactory bulbs, peduncle and tubercle; the diagonal band nucleus and preoptic region; the septal region and amygdaloid nuclei.

The olfactory and accessory olfactory formations have the typical appearance. In the anterior olfactory nucleus a pars ventralis, pars lateralis, pars dorsalis, pars medialis and pars posterior can be recognized, as well as a pars externa. The prominent olfactory tubercle has the usual plexiform, pyramidal and polymorph layers among which are scattered the islands of Calleja.

A broad solid septum on sectioning reveals medial and lateral septal nuclei. The triangular nucleus of Cajal, septo-fimbrial nucleus and bed nuclei of anterior and hippocampal commissures can also be recognized.

A prominent nucleus of the diagonal band is present. Its relation to the preoptic region has been examined in detail. Recent proposals for a more exact definition of this region will be discussed.

Lateral, basal cortical, medial and central amygdaloid nuclei are present, as well as a nucleus of the lateral olfactory tract, intercalated cell masses and an anterior amygdaloid area. A separate accessory basal amygdaloid nucleus cannot however be recognized. The disagreement in the literature about the exact homology of this cell group will be discussed and a solution proposed.

The present findings suggest that phylogenetically the cortical amygdaloid nucleus should be classified with the basal and lateral amygdaloid nuclei, a view which conflicts with that usually accepted.

The centres described, with the exception of the amygdaloid complex (described by Volsch, 1910, in the lemur), have not hitherto been examined in so simple a primate.

JUNE 1951

The Summer Meeting of the Society for the Session 1950–51 was held on Friday and Saturday, 29 and 30 June 1951, in the Medical Building, University College, Cork. The President (Prof. M. F. Lucas Keene) was in the Chair.

The following are the authors' abstracts of the papers read.

Nuclear changes following section of the glossopharyngeal nerve of the pig.

By M. E. Law. (Introduced by M. A. MacConaill, *University College, Cork*)

The taste fibres of the IXth nerve in the pig form a compact and easily accessible bundle; they pass centrally from the circumvallate papillae of the same side. Section of the bundle at this level has given clear evidence (already reported) that the fibres are axons of the sensory cells in the ganglion of the circumvallate papilla, and that these cells, therefore, may be regarded as the primary neurones in the taste path. In a further series of experiments the sectioned fibres have been traced as far as the secondary neurones in the medullary nucleus. The degenerative changes apparent in this region will be discussed and exhibited.

Cutaneous sensibility in the pinna of the ear.

By G. Weddell and D. C. Sinclair. *University of Oxford*

Attempts to identify specific receptors for cold and warm sensibility in the skin by correlating the results of sensory testing with the underlying histology have so far met with little success. Recent work on the histology of the nerve endings of the cornea has shown that the methods used for staining fine neural plexuses and end-organs need most careful control if the results are to be interpreted successfully. When the skin of the external ear in man is stained by methylene blue and silver in the light of this experience, no organized end-organs can be detected there. The innervation of the pinna indeed resembles that of the ear of the rabbit; there are densely innervated hairs, and apparently no other end-organs apart from a fine network of beaded fibres.

Cold, warm, pain and touch stimuli applied to the skin of the pinna have consistently given the appropriate responses in a number of volunteers. It therefore appears that, in this situation at least, such stimuli can be appreciated without the intermediary of any organized endings. This finding will be briefly discussed in relation to theories of cutaneous sensation.

Representation of extra-ocular muscles in the oculomotor nucleus of the monkey.
By R. Warwick. *University of Manchester*

There is current in most text-books of anatomy and neurology a scheme of representation of the extrinsic ocular muscles in the oculomotor nucleus which is based upon the experimental work of Bernheimer (1897) and an analysis of clinical and comparative data by Brouwer (1918). The chief features of this scheme are:

(*a*) The cell groups innervating individual muscles are superimposed in a rostro-caudal order.

(*b*) The levator palpebrae superioris and rectus superior are represented in the most rostral centres, and are supplied by direct fibres.

(*c*) The central nucleus of Perlia consists of cells innervating the recti mediales and mediates convergence.

(*d*) The rectus inferior, obliquus inferior, and rectus medialis are innervated by fibres at least in part arising in the contralateral nucleus.

A careful study of the acute retrograde changes in the oculomotor nuclei of over sixty monkeys, subjected to oculomotor division and extirpation of extraocular muscles, has produced a scheme of muscle representation which contradicts the classical account in all particulars. Its chief features are:

(*a*) The cell groups innervating individual muscles are largely discrete and are columnar masses running in a rostro-caudal direction, the arrangement of which is upon a roughly dorso-ventral basis.

(*b*) The levator palpebrae superioris and rectus superior are represented caudally, and the fibres of the latter are wholly crossed.

(*c*) The recti mediales are not represented by the central nucleus of Perlia in the monkey.

(*d*) The fibres innervating the rectus inferior, obliquus inferior, and rectus medialis are entirely ipsilateral in origin.

Nerve endings and neuro-epithelia in the developing human labyrinth.
By C. C. D. Shute. *London Hospital Medical College, London*

The otic labyrinth has been studied in a series of human embryos stained with silver by de Castro's method. The development of the terminal innervation follows that of the neuro-epithelium and reflects broadly the phylogenetic history of the various parts of the labyrinth. No confirmation was obtained for Lorente de Nó's finding (1926) in mice that the cells of the apical turn of the cochlear duct are highly differentiated before nerve fibres invade the epithelium.

The cristae acquire their innervation early; that of the lateral semicircular duct retains longest its continuity with the primordial utricular neuro-epithelium. The growth of the utricular macula is complicated by the rotation which occurs before it reaches its definitive position. The part related to the utriculo-saccular partition develops late and is sparsely innervated; it corresponds to Lorente de Nó's area of thin and plexiform fibres. A similar area occurs also in the saccule, in which the original thickened medial wall of the otic vesicle differentiates more slowly into non-specialized epithelium and macula proper. The innervation of the cochlea progresses proximally and distally from the site of the original migration of cochlear cells to the basal turn. The more rapidly growing outer hair-cell region receives a scantier plexus of nerve fibres.

The modifications undergone by the invading nerve fibres are similar in different parts of the labyrinth. At a late stage the lagenar region of the cochlear duct shows in longitudinal section a macula-like structure, which nevertheless is continuous with the developing organ of Corti of the apical turn. A search was made for 'fibres égarées' as described by

Tschernjachiwsky (1929) entering the lumen of the utricle. They occurred fairly frequently in the cochlea in relation to the narrow but richly innervated inner hair-cell portion of the neuro-epithelium, but not elsewhere in the labyrinth.

The periodic acid Schiff procedure for the identification of the hyaluronic acid type of mucopolysaccharide. By D. V. Davies. *St Thomas's Hospital Medical School, London*

Hotchkiss, in his description of the periodic acid Schiff method, characterizes hyaluronic acid as yielding a vigorous reaction in 'spot tests'. No information is given as to the method of preparation or probable degree of purity of his preparation. Others have elaborated the P.A.S. technique using the enzyme hyaluronidase to distinguish the hyaluronic acid type of mucopolysaccharide.

In the present investigation three preparations of hyaluronic acid of varying degrees of purity have been studied and compared with other polysaccharides. Some negative results indicate that hyaluronic acid does not react to the periodic acid Schiff technique. This does not conflict with present knowledge of the chemistry of this substance.

The anatomy of the juxtaglomerular complex in the rat and other species. By P. M. Hartroft and W. Hartroft. *Banting and Best Department of Medical Research, University of Toronto*

The morphology of the juxtaglomerular complex in the rat and other selected species has been reinvestigated using a new stain (Wilson & Hartroft, 1951), specially devised to demonstrate the granules of the afibrillar cells found in the afferent glomerular arterioles of the renal cortex. In the normal rat the granules, which are few and small, characteristically occupy a perinuclear position. When granules are increased in storage phases of activity, they become large and form perinuclear haloes. Under conditions of extreme storage, the entire cell is filled with granules and its outline assumes a spherical, swollen shape. Vacuolation of the afibrillar cells has been observed in conditions of hyperactivity with increased liberation of granules. The circumstances in which these morphological variations have been encountered were described and the probable function of the juxtaglomerular complex briefly discussed.

Correlation of cell morphology and histochemistry in fracture callus. By J. J. Pritchard. *St Mary's Hospital Medical School, London*

The distribution of nucleoproteins, mucopolysaccharides, glycogen, alkaline phosphatase, mitochondria, the Golgi elements and lipoids have been studied in the fracture callus of rats and guinea pigs.

This tissue is ideal for studying the differentiation of the cells and matrices of connective tissue, as all types of cell are present from the most undifferentiated to definitive osteocytes, chondrocytes and fibrocytes.

The intensity of the Feulgen reaction for the desoxy-ribonucleoproteins of the nucleus diminishes progressively as osteoblasts and chondroblasts are differentiated from fibroblast-like precursors. Cytoplasmic basophilia, indicative of ribonucleoproteins, is maximal in definitive osteoblasts and chondroblasts.

Young connective tissue and uncalcified cartilage and bone matrices exhibit metachromasia to basic dyes and give a strongly positive reaction with the periodic-Schiff test, indicating the presence of sulphated mucopolysaccharides. Calcified matrices exhibit metachromasia only after decalcification.

Glycogen is present in mature cartilage cells before calcification, but not in significant amounts in other callus cells.

Differentiating and definitive osteoblasts, mature cartilage cells and preosseus collagen fibres exhibit intense alkaline phosphatase activity. The undifferentiated callus cells show little or no activity.

Long rod-shaped mitochondria are very numerous in undifferentiated callus cells and in definitive osteoblasts. They disintegrate in hypertrophic cartilage cells. The Golgi element undergoes marked hypertrophy during the differentiation of osteoblasts and chondroblasts. Discrete lipoidal droplets were only observed in chondroblasts. The significance of these findings is discussed.

Early Miocene lemuroids from East Africa. By W. E. Le Gros Clark and D. P. Thomas. *University of Oxford*

Apart from one molar tooth from Lower Pliocene deposits of the Siwalik formations, the Miocene lemuroids of East Africa provide the only available evidence of the early geological history of the Lorisiformes. Numerous fragments of jaws and teeth of the genus *Progalago*, collected by the British-Kenya Miocene Expedition, as well as a skull with an endocranial cast, have added to our knowledge of this extinct group of lemurs. The fossil material appears to represent at least three species. The dentition, skull and endocranial cast show certain primitive features, but in general they show a close approximation to those of recent Galaginae.

Cleft palate in snakes. By A. d'A. Bellairs and J. D. Boyd. *London Hospital Medical College, London*

As far as we know, the occurrence of cleft palate has not previously been recorded in reptiles other than turtles. Conditions in four late snake embryos showing this anomaly are now described. Unilateral clefts, in one specimen on the left side, in the other on the right, are present in two sibling embryos of the anaconda, *Eunectes murinus*. A bilateral cleft is shown in an embryo of *Natrix natrix*, and a unilateral cleft in an embryo of *Vipero berus*. One of the anaconda embryos was preserved intact; serial sections of all the other specimens were made. The organ of Jacobson on the affected side shows little essential abnormality in each case, and it is suggested that this organ originates entirely within the frontonasal process. The bearing of these observations on the morphogenesis of the palate and lacrimal duct in amniote vertebrates is discussed.

The growth of the grey seal (*Halichoerus grypus* (Fabricius)) from birth to weaning. By E. C. Amoroso and J. H. Mathews. *Royal Veterinary College, London*

The capture of an adult female and her male pup 3 days after parturition enabled us to follow the growth changes in mother and offspring during the nursing period. Both were surprisingly docile in captivity.

During this period the average daily gain in weight of the pup was 3·3 lb., the weight increasing from 43 lb. on the third day after birth to 92 lb. at the time of weaning, a total of 49 lb. The birth weight of the grey seal is about 30 lb. (26–33 lb.), the males being on the whole slightly heavier than the females. During the same period, the average daily loss in weight of the mother was 6·3 lb., her weight decreasing from 371 to 276 lb., a total loss of 97 lb. In captivity no solid food was taken by either, but small amounts (undetermined) of water, both fresh and salt, were drunk by both.

Life-history data and daily weighings were recorded for thirteen pups at liberty in the nurseries. These data, though not always complete for each individual, were sufficient to reach general conclusions regarding the behaviour and development of the young before they go to sea. It would appear that the growth rate of the captive pup was not materially affected by the conditions of captivity. The captive pup was weaned on the eighteenth day after birth and left the beach 12 days later when it had completed the moult of its natal coat. Most animals lost weight slowly after being deserted.

Samples of milk were obtained daily and the provisional preliminary analysis of the pooled sample showed 67·7 % total solids, 52·2 % fat, 2·6 % lactose, 11·2 % protein and 0·7 % ash. The milk fat is typically similar to the seal blubber fats; it is, however, rather higher in palmatic acid than any seal blubber fats on record.

Several pups killed by rough weather, shortly after birth, were dissected. The condition in the genital tract in the newborn presents several interesting features, one of which is the enormous development of the gonads. Another is the engorgement and hypertrophy of the uterus and vagina so that they resemble the similar organs of the terrier bitch on heat, and a third is the occlusion of the anterior vagina by a plug of mucus. These changes in the genital tract are short-lasting and by the fifth day after birth the interstitial cells have begun to undergo a lipid degeneration, while the amount of mucus in the vagina decreases and its viscosity is lowered. The enlargement which occurs in the ovaries and testes alike, is caused solely by the enormous development of the interstitial cells. These cells are large, and closely resemble the cells in the placenta of the mare which are believed to be implicated in the synthesis of serum gonadotrophin.

Histological examination shows the uterus to be well vascularized. The mucosa is thickened and the uterine glands which are numerous, enlarged and actively secreting, reach to its base. The vaginal epithelium is stratified and in places cornified; at others it is converted into a mucus-secreting type with occasional enlarged, granular, darkly staining cells whose nature has not yet been determined. The secretion which accumulates in the lumen of the vagina contains desquamated epithelial cells in addition to red and white blood corpuscles. The extravasated blood in the vagina is small in amount and insufficient to colour the mucus.

The reaction of the interstitial cells resembles a response to the luteinizing hormone of the anterior pituitary, and a number of facts imply that the placenta represents the new source, though the evidence falls short of finality and depends largely on the demonstration that the curve of growth of the cells coincides with the life span of the placenta. Moreover, the differentiation of the uterus and vagina which normally prevails at birth is, in almost every respect, similar to that induced by progesterone acting on an oestrogen-primed genital tract. And here too, though absolute proof is also lacking, the placenta is believed to have assumed gonadal functions.

A film will be available to demonstrate the methods used in this study and to show points of interest in the nursing behaviour of both mother and pup.

Changes in the reproductive tract of foetal and adult seals. By R. J. Harrison. *Charing Cross Hospital Medical School, London*

The appearance of the ovaries of a number of antarctic and common seals have already been described (Harrison & Matthews, 1950, 1951). The changes in the reproductive tracts of these animals and the genital tracts of several foetuses of the common seal have been examined. The most striking changes occur in the vaginal epithelium during the period of delay of implantation when the superficial layers contain tall columnar cells. After implantation and during early pregnancy the epithelium resembles the transitional type. The uterine mucosa during the period of delay is remarkable for the relative paucity of glands, the frequent presence of cysts, and the absence of marked secretory changes.

It has already been noted in a previous communication (1949) that the ovaries of near-term foetuses of the common seal are far larger than those of the mother. The vaginal epithelium of these foetuses is markedly thickened and shows evidence of desquamation. The uterine mucosa contains numerous glands which show signs of active secretion. The factors causing these changes are discussed.

The interaction of the trophoblast and endometrium at the time of implantation in the sheep. By E. C. Amoroso. *Royal Veterinary College, London*

It is generally believed that the final relationship which the trophoblast acquires with the uterine tissues is determined by the differing qualities of its constituent cells. It is believed, moreover, that most of the endometrium which is destroyed by the growing ovum undergoes extensive cytolysis before being absorbed, as postulated by Prof. J. P. Hill and others, instead of being directly phagocytosed in the form of gross particles.

In the light of these hypotheses, it is of interest to consider the changes which take place in the placenta of the sheep during the early phases of its differentiation. The mode of attachment of foetal and maternal cotyledons in the ewe was studied by Assheton (1906), and the present account is a confirmation of his findings. The chief questions at issue centre around the extent of the denudation of the uterine epithelium and the origin of the lining cells of the maternal crypts. In other words, is the sheep placenta wholly syndesmochorial or is it in part epitheliochorial?

Like Assheton, we believe that the destruction of the uterine epithelium is brought about at first by the agency of darkly staining binucleate cells of the trophoblast. These migrate through the uterine epithelium and come to lie on the underlying stroma. In their passage they become enormously enlarged and give rise to a layer of cells which remains closely connected to the cells of the stroma. In the destruction of the epithelium they are aided by others which are more phagocytic, and which actually engulf portions of the uterine epithelium. By the twentieth day of pregnancy the whole surface of the burr is denuded of uterine epithelium, its place being taken by an irregular and interrupted layer of enlarged multinucleated trophoblast cells. Sometime later this layer is quite continuous, but with the advance of pregnancy it again becomes lost at certain places (e.g. at the tips of the maternal septa). Where the trophoblast is in contact with the surface of the burr it is greatly thickened and its cells are filled with rod-like bodies—the Uterinstäbschen.

In the intercotyledonary area the contact of the foetal trophoblast with the mucosa also results in the erosion of the uterine epithelium. Here large polyhedral cells occur over the abraded surface just as they do over the burrs, and the evidence is all in favour of these cells being of foetal and not maternal origin. The epithelium of the maternal crypts is also regarded as being foetal in origin.

Evidence will be presented for the direct passage of binucleate cells from the trophoblast to the surface of the maternal endometrium, to become, in some cases (sheep) typical syncytial giant cells, in others (cow) simple cuboidal elements. Consequently the 'placental membrane', according to strict definition, and employing Grosser's terminology, is of the syndesmochorial rather than the epitheliochorial type.

The presumptive foregut area in the chick blastoderm. By Ruth Bellairs.
(Introduced by J. Z. Young, *University College, London*)

Chick blastoderms were explanted to the surface of plasma clots (watch-glass method of tissue culture). The ectoderm surface was laid against the clot and, in consequence, carbon marks could be placed on any region of the endoderm. After 1–2 days' incubation the developing embryo was sectioned serially and the foregut examined for presence or absence of carbon. The results of about 350 such experiments suggest that the presumptive foregut area lies in the region of the anterior end of the primitive streak and of the head process.

The placodal relations of the neural crest in the domestic cat. By G. Halley.
Royal Veterinary College, London, and Department of Veterinary Anatomy, Bristol

Three separate masses of neural crest material appear in the head region of embryos of the domestic cat, the rostral, the acoustico-facial, and the vago-glossopharyngeal crests.

Each of these masses comes into contact with proliferative areas of the lateral somatic ectoderm at some stage before the embryo has attained a C.R. length of 10 mm. These areas of proliferative ectoderm are the epibranchial and dorsolateral placodes.

Histological evidence suggests that these placodes contribute towards the formation of the geniculate (VII), the petrosal (IX), the nodose (X) and possibly the trigeminal (V) ganglia. The acoustic ganglion (VIII) arises largely, if not entirely, from the auditory placode.

The placodal relations of the nodose ganglion of the vagus nerve play a major role in the developmental fate of the cervical sinus.

The nature of the neural crest and its associated ectodermal placodes in embryos of five somites to 10 mm. was discussed in relation to the general morphology of the developing head.

The development of the sinu-atrial node in the heart of the sheep.
By A. R. Muir. *University of Edinburgh*

In a recent account of the development of the conducting tissue in the sheep Field (1951) was unable to distinguish histological differentiation of the sinu-atrial node until the 100 mm. stage.

The hearts of forty pre-natal sheep have been examined, and significant differentiation has been observed at 10·6 mm. This supports the observations of Sanabria (1936), who found a thickening of the sinu-atrial junction at 7 mm. and demonstrated histological differentiation at 10 mm.

The early nodal cells are paler than the atrial muscle-cells and their nuclei are more spherical and more chromatic. These characteristics can be observed throughout prenatal life, and with the increase in size of the atrial muscle cell the compact group of nuclei of the node becomes more obvious. Nerve cells can be seen in relation to the node at the 44 mm. stage, and in all the later specimens the presence of clusters of nerve cells and groups of nerve fibres is one of the most characteristic features of the node. The 'artery of the node' can be recognized at 25 mm. and is a constant and definite guide to the node in the older embryos.

The main part of the node is first recognized in its definitive position at the cephalic end of the sinu-atrial junction. The prolongation of the node into the interatrial septum can be observed as soon as the septum secundum is formed, at the 71 mm. stage. An extension from the right edge of the node can be traced caudally in the base of the right venous valve (crista terminalis) at the 155 mm. stage.

The innervation of the kidney. By Z. M. El-Asfoury.
London Hospital Medical College, London

The richness of the collagen and reticular fibres and of the cementing substance in the kidney may be a partial explanation of the difficulty in staining nerve fibres in it. Specific enzymes (collagenase and hyaluronidase) have been used in an attempt to overcome this difficulty. By various techniques and especially the freeze-drying method, myelinated and unmyelinated nerve fibres have been found inside the kidney. Endings of these fibres and possible synapses are described. Observations are also recorded on the distribution of nerve cells in the kidney between the tubules, inside the glomeruli and along the blood vessels. The findings after renal denervation confirm these observations.

Post-natal patency of the umbilical vein. By H. Butler.
St Bartholomew's Hospital Medical College, London

Large anastomotic vessels connecting the portal and epigastric veins are frequently found in the falciform ligament in cases of portal venous obstruction. They have been variously regarded as:

(*a*) Direct communications between the portal and epigastric veins (the para-umbilical veins of Sappey, 1889).

(*b*) Congenital patency of the umbilical vein (Cruveilhier, 1842, Baumgarten, 1891).

(*c*) A combination of the dilated residual lumen of the umbilical vein in the hepatic end of the ligamentum teres (the 'Rest Kanal' of Baumgarten, 1891) and the veins of Burow (1838), (Eppinger, 1920; Hanganutz, 1922).

(*d*) Recanalization of the obliterated umbilical vein (Wertheimer, 1886).

Examination of the microscopic structure of five such veins from cases of portal venous obstruction and of ten specimens of normal ligamentum teres from individuals ranging in age from 2 days to 77 years shows that:

(*a*) A much reduced, but patent residual lumen of the umbilical vein is frequently present along the entire length of the normal ligamentum teres. It communicates directly with the left branch of the portal vein and with the epigastric veins via the veins of Burow.

(*b*) After birth the lumen of the partially contracted umbilical vein is further reduced by collapse of the vessel wall followed by hyperplasia of the intima. There is no evidence of the occurrence of thrombosis followed by organization and the formation of new channels.

(*c*) During post-natal life this lumen becomes surrounded by numerous longitudinal fasciculi of smooth muscle. In the full-term foetus and in the newborn infant there exists but a scant quantity of spirally arranged smooth muscle in the wall of the intra-abdominal umbilical vein. External to the longitudinal smooth muscle a thick layer of white fibrous tissue is laid down.

(*d*) In portal venous obstruction the residual lumen of the umbilical vein may become increased to a diameter greater than that obtaining in the full-term foetus. This phenomenon is accompanied by a commensurate increase in the size of the veins of Burow.

The mechanism of attachment of the blastocyst in the cat.

By C. B. Murray. *Royal Veterinary College, London*

By the thirteenth day of pregnancy each blastocyst is found lying free in a spherical dilatation of the main uterine lumen. But between the thirteenth and the fourteenth day of pregnancy the trophoblast, in an annular zone encircling the equator of the oval sac, invades the endometrium, so that henceforth a true placental relationship is established. The area of attachment is at first restricted to the trophoblast surrounding the embryonal area, but rapidly extends as a girdle around the central portions of the blastodermic vesicle. However, a complete band of attachment is not produced until the amnion folds have fused.

At the time of attachment the endometrium in the placental zone is predominantly a glandular structure and structural differences in the various parts of the glands cause it to appear laminated. The basal half of each gland forms a small coiled tube, whereas the superficial half is dilated and forms a uterine crypt.

Prior to attachment the trophoblast is cellular, but even before attachment is completed it becomes differentiated into a basal cytotrophoblast and a superficial syncytial layer. Evidence will be presented to show the manner of growth and differentiation of the attaching trophoblast.

Encephalon bifidum. By W. R. M. Morton. *Queen's University, Belfast*

Studies of four embryos showing non-closure of the anterior neuropore have been made. The material consists of a 20 mm. c.r. length bovine embryo, and three embryos corresponding in general development to those of Streeter's age-groups 13 and 14 (4–5 mm. and 5–8 mm. c.r. approx.) and a normal human embryo of 30 mm. c.r. length.

The smallest human embryo shows a very slight eversion of the neural tissue of the forebrain through a still widely open anterior neuropore. The next larger human embryo exhibits a more widely open anterior neuropore with gross eversion of the neural plate tissue of fore- and mid-brain. There is a sharp line of demarcation between the contiguous neural and skin ectoderm in both these cases, and differentiation of cell layers in the everted tissue of the larger embryo is considerable. The 20 mm. c.r. bovine embryo shows a smaller defect in the region of the mid-brain only. The largest embryo, corresponding to 30 mm. c.r. length (23 mm. actual c.r. length), shows a widely open defect of the brain with almost complete solution of continuity of the brain tissue in the hind-brain region; it is in fact almost a true anencephalic embryo.

The changes seen in the brains of these embryos illustrate the probable sequence of events occurring in the exposed brain tissue of defective embryos which leads up to frank anencephaly. Failure of the lips of the medullary plate to meet, fuse, and separate off from the skin leads to a lack of tension medially and an abnormal persistence of tension laterally between the skin and the neural ectoderm. The continued growth of the brain tissue at a greater rate than that of the skin leads to an eversion of the neural plate in a manner similar to that by which reversal of the embryonic shield occurs, instead of to the normal folding of the brain; although folding does occur in the normal parts of the brain where the

defect is small. Finally, erosion of the exposed brain tissue occurs after most or all of the cranial nerves have become independent of the brain for the continuity of their existence. There is no evidence here concerning the underlying cause of the non-closure, but defective nutrition could have been a contributory factor in the three human specimens (two ectopics and one abortion). Nothing is known concerning the uterine conditions of the bovine specimen.

Opposition of the human thumb.

By J. R. Napier.
St Thomas's Hospital Medical School, London

Investigation of the late results of injuries to the median nerve in man (sixty consecutive cases) reveals that normal opposition (defined as the position of the thumb, produced by a combination of movements of the carpo-metacarpal, metacarpo-phalangeal and inter-phalangeal joints, which allows each remaining digit to be brought into effective pulp-to-pulp contact with its terminal phalanx) is seldom regained even in the presence of good recovery of muscle power. The failure to produce opposition of the thumb in these cases is due mainly to the loss of abduction and medial rotation of the metacarpo-phalangeal joint, a movement normally produced by the abductor pollicis brevis muscle which acts principally on this joint. In addition the abductor pollicis brevis produces abduction of the metacarpal at right angles to the palm in association with the abductor pollicis longus, the opponens pollicis and the flexor brevis. Loss of abduction at the carpo-metacarpal joint therefore does not usually occur in an isolated paralysis of abductor pollicis brevis; in these cases, however, the imperfect opposition, which always results, is attributable to the absence of the abduction-rotation movement at the metacarpo-phalangeal joint.

The importance of this movement does not appear to be generally recognized, although it has been fully described by Duchenne (1867) and Du Bois Reymond (1896) and more recently by Bunnell (1948). The anatomical basis of this clinical observation has been investigated in the normal subject by dissection (twenty limbs), radiological examination and electrical stimulation, and these findings are discussed.

The mechanism of the ankle joint.

By T. J. Harrison.
Queen's University, Belfast

This investigation was undertaken to find out if any rotational change occurred in the relations of the tibia and fibula during movements of the ankle joint. The ligaments at the lower end of the tibia and fibula were examined histologically for the presence of elastic fibres.

Six amputated legs and one living leg were used in the investigation. Kirschner's wires were inserted into the tibia and fibula in order to show the degrees of rotation, and X-ray films and photographs taken with the foot in various positions. In the amputated legs wires were also inserted into the talus to observe its relation to the leg bones during movement.

It was found that when the foot was adducted and passed from the plantar-flexed to the dorsi-flexed position medial rotation of the tibia in relation to the fibula was of 2–4°, and occurred in all the legs examined. During this movement fibula and talus maintained fairly constant relationship. With the foot abducted and passed from plantar-flexion to dorsi-flexion, rotation between tibia and fibula varied. It was always less in degree than when the foot was adducted in any particular specimen, and occasionally it did not occur. The talus during this movement rotated first laterally and then medially around a vertical axis in relation to the tibia. It is suggested that this last movement of the talus in dorsi-flexion of the ankle joint is of significance in the mechanism of locomotion, and causes a lateral rotation of the tibia when the talus is relatively fixed at the subtalar joint.

The medial ligament of the ankle joint, especially that part attached to the navicular and plantar calcaneo-navicular ligament, was the only ligament found to contain significant amounts of elastic tissue.

Further observations concerning erectores spinae function in trunk flexion. By W. F. Floyd and P. H. S. Silver. *Middlesex Hospital Medical School, London*

The function of the erectores spinae has been studied so far in eighty human subjects, using various electromyographic techniques.

As previously reported (Floyd & Silver, *Lancet*, 1951, 1, 133–4) the erectores spinae relax when the trunk is fully flexed. The mechanism of this relaxation in trunk flexion has now been investigated. The following evidence has been collected:

(1) Increased tension in the ligamenta flava and interspinous ligaments in the monkey did not produce reflex inhibition of the erectores spinae.

(2) Relaxation occurred in the absence of tension in one or two intervertebral segments in patients after spinal fusion.

(3) Relaxation occurred in a patient whose vertebral column was ankylosed, without lengthening of the muscle fibres.

(4) The orientation of the vestibular apparatus was without effect.

(5) The position of flexion when relaxation occurred was studied with simultaneous radiography and electromyography. A greater degree of flexion was observed at the moment of relaxation in standing subjects bending down to the ground than in the same subjects in a position of full flexion in sitting.

(6) Relaxation in full flexion in certain patients occurred normally in sitting but not in standing.

(7) The erectores spinae may remain relaxed during the initial movement of lifting weights up to 56 lbs.

It is suggested that erectores spinae relaxation reflexly accompanies the transfer of strain from muscle to ligament, and is achieved through proprioceptive pathways.

The meningeal arteries in man. By M. J. Waas. (Introduced by G. A. G. Mitchell, *University of Manchester*)

A detailed study has been made of the sources and distribution of the anterior and posterior meningeal arteries, and of the position of the primary division and types of branching of the middle meningeal artery. The investigation has been carried out in fifty still-born infants using various techniques including dissection after arterial injection, radiography, histological methods and clearing techniques of the Spalteholtz type.

The following observations were made amongst others:

(1) A constant anterior median meningeal branch, passing backwards in the roof of the superior sagittal sinus, is usually derived from one or both anterior ethmoidal arteries; occasionally it is a branch of the anterior cerebral artery.

(2) There are four common types of branching of the middle meningeal artery, and these and their relative position and frequency were described.

(3) The posterior meningeal vessels showed a variety of complex arrangements. The common patterns were described.

(4) The falx cerebri is constantly supplied by the ethmoidal and middle meningeal arteries, variably reinforced by branches from the anterior cerebral and rarely from the superior cerebellar.

(5) The tentorium cerebelli receives a constant supply from the internal carotid and middle meningeal arteries, reinforced by the meningeal branches of the occipital and vertebral arteries. The supply from the internal carotid is sometimes replaced by a supply from the superior cerebellar.

Cyclical changes in the uterine mucosa and vagina of the goat. By W. J. Hamilton and R. J. Harrison. *Charing Cross Hospital Medical School, London*

The changes in the reproductive tract have been examined in a series of seventeen goats killed at known times during the cycle. The appearances of the ovaries of these animals have already been described (Harrison, 1948). Certain histological changes are found to be abrupt in onset and short in duration. The most pronounced occur in the vaginal epithelium

during the first few days of the cycle. A sudden appearance of cornification and subsequent rapid desquamation would indicate an intense heat of short duration. The quiescent state of the vaginal epithelium during the remainder of the cycle is interrupted around the twelfth day by the temporary appearance of a transitional epithelium. The changes in the uterine horn and cervix do not present such contrasts. The epithelium and the glands show maximal secretory activity from the 9th–13th days of the cycle. Thereafter, there is a steady decline in activity which corresponds closely to the retrogressive changes in the corpus luteum. The changes in the uterus are essentially similar to those described by Corner (1921) for the sow, and can be closely correlated with those in the cow (Cole, 1930).

Observations on the development of the muscularis mucosae in the human gastro-intestinal tract. By INDARJIT. *London Hospital Medical College, London*

The development of the muscularis mucosae in the various parts of the gastro-intestinal tract has been studied in a large number of embryos and foetuses. The muscularis mucosae does not follow the same sequence of development as the muscular coats of the gut. In the oesophagus and the duodenum the order of development is caudo-cranial. In the jejunum and the ileum, it develops almost simultaneously. The time and the order of the appearance of the two layers are discussed.

The cartilage of the nasal septum and facial growth.
By J. H. SCOTT. *Queen's University, Belfast*

The parts making up the nasal septum have been investigated in members of the chief mammalian orders. In all cases the septum consists of the vomer, a perpendicular plate, which may be derived from the presphenoid or from a separate centre of ossification, the mesethmoid, and the septal cartilage. The perpendicular plate extends forwards by a process of endochondral ossification of the back of the septal cartilage. The relations of the cartilage to the other bones with which it articulates are such as to permit separation of the bones at the facial sutures as a result of the interstitial growth of the cartilage. It is this separation of the bones which permits growth to take place at the sutures. The evidence indicates that growth at the anterior facial sutures (between the premaxillary, nasal and maxillary bones) continues for a considerable time after growth has ceased at the sutures behind the maxilla (posterior facial sutures). The ethmoid consists of two developmentally and functionally distinct parts; the perpendicular plate which is part of the cranial skeleton, and the lateral (turbinate) masses which are parts of the facial skeleton. The two parts unite some time after birth with the extension of ossification across the cribriform plate, and this union terminates the foetal and immediate postnatal period during which the facial skeleton is thrust forwards from the cranial base as a result of interstitial growth of the septal cartilage.

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